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1985

NATIONAL INSTITUTE OF DENTAL
RESEARCH (U.S.)

ANNUAL REPORT OF INTRAMURAL
RESEARCH, FISCAL YEAR 1985

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National Institute of Dental Research (U.S.)

ANNUAL REPORT of Intramural Research

Fiscal Year 1985

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Services
National Institutes of Health
National Institute of Dental Research

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1985

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EPIDEMIOLOGY AND ORAL DISEASES PREVENTION PROGRAM

ANNUAL REPORT OF THE ASSOCIATE DIRECTOR
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH

The major new research emphasis of the E&ODP Program this year was in the area of the epidemiology of periodontal disease.

At present, there is no reliable method to detect individuals at high risk of developing periodontal disease, though such a screening method would be especially useful in devising prevention strategies. A longitudinal study of adolescents was begun by Program staff to establish the prevalence of incipient loss of attachment of the periodontal soft tissues and loss of marginal alveolar bone in young persons and, by annual follow-up, to attempt to identify patterns of early signs of disease which may be predictive of later, destructive periodontitis.

It has been a frequent clinical observation that many older persons remain free from periodontitis despite the presence of known risk factors such as age, bacterial plaque and calculus. Much remains to be learned about the role of specific microorganisms and host defense mechanisms in the etiology of the disease. To more systematically study these factors we began a collaborative study with two universities in which adults with and wihout destructive periodontal disease will be compared in an intensive evaluation with a battery of microbiologic, biochemical and immunologic assays, as well as detailed medical and personal histories.

Selective resistance to periodontal disease can also be demonstrated by epidemiologic methods. A fifteen-year study was completed of an adult male population in Sri Lanka who had no access to dental care. The results clearly showed that although the group as a whole was highly susceptible to chronic periodontal disease, one subset had disease of the rapidly advancing type, while a small percentage of subjects remained essentially disease-free throughout the period of observation. Evidently efficient future efforts to control this disease will require some method of targeting preventive efforts to the highest risk groups in a population.

Both epidemiologic studies and therapeutic clinical trials of periodontal diseases are hindered by the lack of rational, generally-accepted methods for clinical description of disease prevalence and of statistical methodology for analysis of both cross-sectional and longitudinal data. A new method was developed and published for the summary and analysis of epidemiologic data on the prevalence of periodontal attachment loss, and studies were initiated to develop new techniques for analysis of time series of multiple, repeated measurements of attachment level, of the kind collected in clinical trials.

Studies of improved methods for caries prevention with topical fluorides continued. A Phase II clinical trial of intraoral controlled release device was successfully completed. The results showed that release of fluoride can be regulated within pre-specified limits for periods up to 6 months, resulting in plaque-fluoride levels which are, presumably, substantially above those required for caries prevention. A long-term trial of the device as a caries-prevention technique is now planned. In other animal and pilot human studies it was demonstrated that dicalcium diphosphate, when used together with stannous fluoride, significantly increases uptake of fluoride by enamel without interfering with the antibicrobial activity of SnF. This combination thus has potential for simultaneously protecting against caries and gingival disease, and will be tested further in humans.

As the use of fluorides in various forms for caries prevention becomes increasingly common in the U.S., the need for careful monitoring of total fluoride consumption increases. Fluorosis of tooth enamel is the most sensitive clinical indicator of excessive fluoride intake. Program staff conducted a second study of the prevalence of fluorosis in several communities in Illinois with varying levels of naturally-occuring fluoride in their water supplies. These data are still being analyzed, but thus far indicate no noteworthy increases in fluorosis compared to a previous survey in 1982 or to baseline data reported over 40 years earlier. Nevertheless, we intend to continue periodic surveillance of this situation.

Substantial effort was given to the initiation and monitoring of a comprehensive national epidemiologic survey of the oral health of adults. The study, despite numerous logistical problems, is proceeding on schedule and data collection will be complete in December, 1985. The resultant analyses are expected to provide the most precise estimates ever available on the prevalence and distribution of coronal caries, root-surface caries and periodontal disease in U.S. adults.

In 1980, the results of a nation-wide epidemiologic study of dental caries in school children showed that the disease had undergone a dramatic decline in prevalence during the preceeding eight-year period. Whether this trend is continuing is a critically important question to the planning of future directions in dental reseach, prevention and education. Accordingly, we developed, during the year, a protocol for a second epidemiologic survey of U.S. school children, to begin in September 1986. In addition to data on dental caries, national estimates will also be made of the prevalence of enamel fluorosis and of early signs of periodontal disease in adolescents.

The Program organized and convened an international workshop on Guidelines for Epidemiological Research in Temporomandibular Disorders in an attempt to bring some order and diagnostic consistency to this very .pa confused, but expanding, field of research. Some agreement was reached regarding the constellation of signs and symptoms necessary for differential diagnosis of this syndrome, and the possible ways in which they can be measured.

The Associate Director was appointed to the Surgeon General's Advisory Committee on the Health Consequences of Using Smokeless Tobacco, served on the organizing committee for an NIH Consensus Development Conference on this topic and was elected for a third term to the Executive Board of the European Organization for Caries Research. At the request of the latter organization, a short course was developed and organized titled: "Epidemiology: Methods and Applications." The course will be offered to young American and European dental researchers in Summer, 1986.

Program management was seriously disrupted by the sudden death of Dr. Jerry Niswander, Chief of the Epidemiology Branch. Recruiting to fill this key position has not yet been successful. During the year, Dr. Cyril Enwonwu from Nigeria and Dr. Jukka Ainamo from Finland joined the Program as Visiting Scientists. Ms. Rhea Meyers retired after 14 years of service in the Clinical Trials Section, Disease Prevention Branch.

ANNUAL REPORT OF THE BIOMETRY SECTION

NATIONAL INSTITUTE OF DENTAL RESEARCH

All members of the Biometry Section were involved in preparation for and implementation of the National Survey of Adult Oral Health. In conjunction with members of the Field Studies and Clinical Trials Sections, indices to measure coronal and root surface caries and periodontal destruction were designed, tested and documented. Forms for efficient, accurate data collection of the indices were designed and printed. Training and calibration of contractor staff of dentists and recorders took place in January 1985. Analysis of the calibration exams was done on site to provide feedback to the examiners and the standard to facilitate correction of diagnostic problems. Results of the calibration exams indicated very good agreement on measurements of caries among all dentists and good agreement between individual dentists and the standard examiners on measurements of periodontal destruction. Exams were begun in February 1985 and are expected to be completed by December, 1985.

A new optical character reader was installed within the Biometry area for easier access and control of data processing. A communication program to transfer data directly to the central computer at NIH from the scanner was designed and implemented. New computer programs to process and edit the specially designed forms of the survey as well as standard forms were written and tested.

Statistical methodology for assessing the reliability of an ordinally scaled index was investigated using the PGMI index for gingival inflammation as measured by five examiners. Interclass correlation coefficients and weighted kappa statistics for establishing and examining examiner agreement were utilized. These types of procedures can be useful for training and calibrating multiple examiners who will be using such indices in clinical studies. This material was presented at a conference on Periodontal Clinical Trials in May.

Research has been initiated into better analysis of incomplete longitudinal dental data. A new procedure to allow an investigator to draw an overall conclusion as to whether the treatment group is better than the control group over the entire study period will be tested on caries data from numerous clinical trials where children may have missed one or more exams. A special computer program has been acquired and is being tested.

In addition to individual research, statisticians in the Biometry Section did extensive work with other investigators on analysis of periodontal destruction both from a long-term study of the natural history of periodontal disease and cross-sectional exams of loss of attachment. New summary statistics were generated and evaluated.

A large-scale study of reliability of the feeding machine animal model for evaluating cariogenicity of foodstuffs was designed and analyzed in collaboration with the Laboratory Methods Section. This material will be presented at a Diet and Nutrition Workshop to be held in October 1985.

Further analysis of change in certain microorganisms in saliva and their relationship to caries prevalence and incidence in teenagers was investigated in conjunction with a clinical trial being conducted by the University of Michigan. A positive correlation between the level of S. mutans and Lactobacillus in saliva and the incidence and prevalence of dental caries in this population was confirmed. This material was presented at the IADR meeting in March.

Biometry members collaborated with the Laboratory Methods Section in a study of the influence of water hardness and fluoride on dental caries. DMFS and fluorosis exams were conducted in five communities in Texas. The relationship of fluorosis and level of fluoride in the drinking water of life-long residents was reaffirmed.

Statistical assistants also participated in data collection in a fluorosis study in Illinois and training and calibration for the adult oral health survey. Data, both caries and other measurements, were processed, edited and tabulated from four intramural studies and five contracted clinical trials. Data from numerous rat studies were collected and standard tabulations and analysis done.

A major portion of time was allocated to processing and editing the second phase of the National Center for Health Statistics (NCHS) Hispanic HANES survey. This consisted of approximately 5000 dental exams conducted in New York, New Jersey and Florida.

During this year Biometry members participated in three major conferences: periodontal clinical trials, oral hygiene and plaque and temporomandibular joint problems. They continued active participation on the NIH Epidemiology Committee and the Task Force on Design and Analysis of Clinical Trials. Statisticians acted as consultants to the naval dental school in Bethesda and the VA dental materials project.

Consultation on design of animal and human studies was given to members of all sections of the E&ODP Program as well as other investigators in the NIDR intramural and extramural programs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00292-06 EODP

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of the National Dental Caries Prevalence Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

BRUNELLE, J.A.	CHIEF, BIOMETRY SECTION	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A nationwide survey to assess the prevalence of dental caries throughout the United States was designed and implemented during the 1979-80 school year. A probability sample of school-aged children in grades kindergarten through twelve was selected for examination for dental caries, gingivitis, and need for restorative treatment. Estimates of disease level and treatment need were made for the continental U.S. and each of seven geographic regions by age, race and sex. Additional analyses such as patterns of eruption, caries susceptibility by surface type and caries prevalence by residential fluoride history were calculated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00353-04

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A More Efficient Use of Initial DMFS in Predicting Caries Incidence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

KINGMAN, ALBERT STATISTICIAN EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS: .01	PROFESSIONAL: .01	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prediction models for the development of dental caries have been investigated by many researchers. To date, the variables shown most often to be correlated with the development of dental caries are the subject's DMFS prevalence and the number of permanent surfaces that exist in the dentition. In a previous study, Kingman has shown that the incorporation of the various surface susceptibility levels for caries into the subject's caries prevalence assessment can improve models used for caries prediction.

The current investigation focuses on differences in surface susceptibility levels to caries attack and also the separate counts of Dm F and M surfaces diagnosed for the subject. A weighted caries experience is derived using these factors and tested for its predictive power, using recently conducted clinical trial data sets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00354-04

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods of Analyzing Microbiological Changes in Plaque

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

LI, SHOU-HUA STATISTICIAN (HEALTH) EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.20	.20	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three statistical methods of analyzing repeated measurements on microbiological changes in plaque were investigated. The simplest method to use is the univariate analysis of variance. This approach has an exact F distribution when hypotheses are tested under restrictive assumptions of normality and equal variances and covariances. An alternative approach called profile analysis is based on the multivariate analysis of variance technique. In the profile analysis, the readings on each subject at t times are regarded as a t-dimensional vector. A third approach is based on the polynomial growth curve model, i.e., a separate polynomial growth model is fit for each group of data.

Data from an eight-week study on the natural transmission of S. mutans in rats on five different dietary concentrations of sucrose was used to illustrate these methods. Statistical analysis indicated that the assumption of equal variance and covariance in the univariate analysis of variance was violated. It was shown that different sucrose concentrations in the diets produced significantly different S. mutans growth profiles. Each sucrose group over time was summarized by a single polynomial equation of time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00387-02

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Relationship Between Specific Microorganisms in Saliva and Dental Caries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

KINGMAN, ALBERT	STATISTICIAN	EB, EODPP, NIDR
SHEATS, ROSE	STAFF FELLOW	EB, EODPP, NIDR
LITTLE, WAYNE	MICROBIOLOGIST	EB, EODPP, NIDR
GOMEZ, IRMA	MICROBIOLOGIST	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.22	.02	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Potential associations between the levels of specific micro-organisms in saliva and the level of dental caries prevalence and incidence in a U.S. adolescent population are being investigated. Initial and interim findings indicate that there is a positive correlation between the level of S. mutans and Lactobacillus in saliva and the prevalence and incidence of dental caries in this study population. The initial caries prevalence for participants having high levels of bacteria in saliva were 1.5 DMF surfaces higher than that of those having low levels of bacteria in their saliva. There were 1.1 fewer new DMF surfaces that developed in subjects with low levels of bacteria in their saliva compared with those having high levels of bacteria in their saliva during the first half of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00388-02

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of a New Surface Index of Fluorosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

KINGMAN, ALBERT	STATISTICIAN	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new index for scoring dental fluorosis, denoted by TSIF, recently developed at the NIDR, was evaluated for reproducibility by the two examiners who performed the fluorosis examinations. Each examiner was able to reproduce his fluorosis diagnosis upon repeated evaluation of a subsample of participants. The examiners experienced some difficulty in agreeing between themselves, especially in making the differentiation between the presence or absence of the very mild form of fluorosis.

In spite of the difference between examiners, this index was able to detect statistically different levels of fluorosis between groups having optimal, 2, 3 and 4 times optimal levels of fluoride in their water supplies, after adjusting for examiner differences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00389-02 EODP

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tooth Eruption Patterns of U.S. Schoolchildren, 1979-80

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

SMITH, JACQUELINE I.	STATISTICIAN	EODPP, NIDR
BRUNELLE, JANET A.	CHIEF, BIOMETRY SECTION	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.02	.02	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Caries attack rates for permanent teeth of children are greatly influenced by the duration of exposure to risk. To better access caries susceptibility, it was decided to determine the eruption patterns of the children being studied. Using the dental findings of 37,066 children examined during the 1979-80 National Dental Caries Prevalence Survey, an estimation of the time of eruption for permanent teeth was made for children ages 4-18. Calculations were made of the percentages of children having each tooth at each age. Ages for the survey were rounded to the nearest birthdate. Eruption patterns were calculated for each upper and lower tooth by sex and race. Probit analysis were used to establish time of eruption and corresponding confidence limits.

The age at eruption of mandibular teeth was usually earlier than maxillary teeth, though some teeth, such as 2nd bicuspids, erupted at about the same time in both arches. As expected, there is a definite hierarchy of eruption by race and sex, with teeth erupting earlier in females than in males and in blacks earlier than in whites. The largest difference in age of eruption occurred in upper and lower cuspids, which erupted approximately one and a quarter years earlier in black females than in white males.

Comparing our results with past studies of white children in the U.S., it appears that the teeth of present day white children are erupting earlier.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DE-00402-01

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistical Analysis of Incomplete Longitudinal Dental Data

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

LI, SHOU-HUA	STATISTICIAN (HEALTH)	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
EPIDEMIOLOGY

SECTION
BIOMETRY

INSTITUTE AND LOCATION
NIDR,NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.25	.25	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Much of the literature on the analysis of longitudinal data assumes that the data is complete (no observations are missing in the repeated measurements of the same individual). Yet longitudinal studies typically have missing data. Recent research has focused on the development of statistical methods that will permit the complexities of typical longitudinal data set. Wei and Johnson (1985) have proposed a procedure to allow the investigator to draw an overall conclusion whether the new treatment group constitutes an improvement over the control group for the entire study period. The test procedure allows different patterns of missing observations for the two groups to be compared. This new statistical method will be investigated and illustrated by the analysis of incomplete longitudinal dental data.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00403-01

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Collection and Analysis of Oral Health of U.S. Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

BRUNELLE, JANET A.	CHIEF, BIOMETRY SECTION	EB, EODPP, NIDR
MILLER, ANN J.	EPIDEMIOLOGIST	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
EPIDEMIOLOGY

SECTION
BIOMETRY and FIELD STUDIES

INSTITUTE AND LOCATION
NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.17	.95	1.22

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An epidemiologic survey of oral health in adults was implemented during FY 85. Dental exams are being conducted on a cross-sectional sample, stratified by region and age, of approximately 20,000 employed adults 18 years of age and older and non-institutionalized seniors 65 years of age and over who attend multi-purpose senior centers. These exams will be used to establish the prevalence of coronal caries, root surface caries and periodontal destruction and to estimate the treatment needs of adults in the U.S.

Training of field teams was held in January 1985. Field exams commenced in February and are scheduled to be completed by December 1985.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00409-01

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Procedure for Evaluating the Reliability of a Gingivitis Index

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

KINGMAN, ALBERT STATISTICIAN EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS: .30	PROFESSIONAL: .30	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A methodology is presented for assessing the reliability of an ordinally-scaled index and is illustrated by using data from a clinical trial in which gingival inflammation was assessed with the PMGI index, independently, by five examiners. One of the examiners was an experienced examiner, the others newly trained. All subjects were evaluated by each examiner initially and at the end of the study period. The reliability of the average score per subject, maximum score per subject, and the percentage of affected sites per person are estimated by the intraclass correlation coefficient. Procedures are presented that utilize various forms of the weighted kappa statistic for dissecting patterns in examiner agreement for specific sites, types of sites, all sites, and for the individual components and categories of the index. It is shown how these procedures can be useful for training and calibrating multiple examiners, who will be using such an index in a clinical study, so that adequate reliability levels can be realized.

ANNUAL REPORT OF THE FIELD STUDIES SECTION

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Field Studies Section is charged with conducting epidemiologic studies of oral diseases and conditions, with a current emphasis in the epidemiology of periodontal disease. Other activities of the Section include the monitoring of research contracts in the area of caries prevention as a continuation of work that was initiated before the Section was formally created.

It has been frequently observed that there is a lack of up-to-date nationwide epidemiological data to document the prevalence and severity of dental caries and periodontal disease among American adults. The need for such data increases as a larger proportion of the population becomes older and tooth retention increases. To address this need, a national cross-sectional survey of adult dental health is being conducted in collaboration with a private contractor (Westat Corporation, Rockville, MD). Based on a sample of approximately 20,000 working adults and non-institutionalized senior citizens, the survey will document the prevalence of coronal and root caries and will also assess periodontal health. The survey is ongoing and will be completed in 1986.

Reports on adolescent populations outside the U.S. show a wide variation in the prevalence of periodontal diseases; few reports exist on American adolescents. To assess the periodontal health of this important age group, the Section conducted a cross -sectional study to determine the prevalence of gingivitis, epithelial attachment loss, and bone loss in a group of adolescents residing in New Mexico. The study population consisted of approximately 600 Navajos, 14-19 years of age. Twenty-four posterior interproximal sites were examined on each subject. Preliminary analyses show a high prevalence of disease: 71 percent of subjects had gingivitis, 89 percent had one or more sites with attachment loss, and 89 percent showed evidence of bone loss as measured radiographically. More detailed analyses are being done, and a longitudinal study is planned with younger subjects to investigate microbiologic, systemic and other factors that may contribute to the high prevalence of disease.

Current methods of summarizing data from epidemiological studies of periodontal disease (such as the PI, PDI, and CPITN) reduce detailed clinical examimination data to one score that summarizes the severity of disease. One current interest is to investigate the possibility of using a different summary statistic that will better characterize the clinical data. The data used for this investigation were collected previously in a longitudinal study of periodontal disease. Data consisted of 56 attachment loss measurements from buccal and mesio-buccal sites of 369 male subjects who were examined twice, three years apart. From these measurements a bi-variate statistic was derived, called the Extent and Severity Index (ESI). The ESI summarizes the average extent of diseased sites within the mouth (E) and the average severity of disease, expressed as milimeters of attachment loss (S). E and S provide independently useful information and can be tested with multivariate statistical procedures.

To add to our understanding of the natural history of periodontal disease, the initiation, rate of progress of periodontal disease and consequent tooth loss and associated microbiologic and immunologic factors are being investigated in a population never exposed to preventive or treatment procedures. Male laborers at two tea plantations in Sri Lanka, 14-31 years of age, comprised the initial study group which was first examined in 1970. Five subsequent examinations have been conducted, the most recent in 1985. A cross-sectional sample was also selected from a third plantation in 1985. Three subpopulations have been identified based on loss of attachment and tooth mortality: subjects with rapid progresion of disease (8%), subjects with moderate progresion (81%), and a group who exhibited no progression beyond gingivitis (11%). Further studies are planned to examine the relationship between rates of attachment loss and tooth mortality, and to investigate bacteriologic and immunologic responses in subpopulations based on disease profiles.

Recognizing the need to develop preventive procedures for adults, a clinical trial was initiated under the contract mechanism in 1983 to study the effect of mouthrinsing with neutral sodium fluoride upon the development of root and coronal caries in a group of employed adults, 18-65 years of age, residing in a non-fluoridated community. The study is scheduled for completion in 1987; interim clinical findings are not yet available.

Another contract project is a three-year clinical trial to evaluate the relative anticaries effectiveness of three fluoride dentifrice formulations. The study is comparing the efficacy of the unsupervised home use of two NaF-MFP dentifrices that have fluoride concentrations of 1000 and 2500 ppm with that of a positive control dentifrice that contains MFP at a fluoride concentration of 1000 ppm. The project is nearing completion. Interim results after two years showed no difference between groups; three-year results will be available in late 1985.

Recent controversy about the value of prenatal fluoride supplements has demonstrated the need for more research on this preventive procedure. A five-year clinical trial was initiated in 1983 to determine the effect of fluoride supplements taken during pregnancy to prevent the development of dental caries in the primary dentition of offspring. Volunteers are randomly assigned to either a treatment or a placebo group for the prenatal phase of the study; offspring of both groups will receive fluoride supplements postnatally. More than 1,200 women have been recrutied in non-fluoridated Portland, Maine. As of September, 1985 about 1,000 babies have been born to study participants. The first examinations for dental caries will be conducted when the children are 3 1/2 years of age, beginning in 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00399-01

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Periodontal diseases in adolescents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Carlos, James P.	Associate Director for EODP, NIDR	NIDR EODP
Wolfe, Mary D.	Epidemiologist	NIDR EODP

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Associate Director; Epidemiology Branch

SECTION
Field Studies

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Reports on adolescent populations outside the U.S. indicate a wide variation in the prevalence of periodontal diseases. The purpose of this cross-sectional study is to determine the prevalence of gingivitis, epithelial attachment loss, and bone loss in a group of adolescents residing in the U.S.

The study population consisted of approximately 600 Navajos, ages 14-19 years. Twenty-four posterior interproximal sites were examined on each subject. Gingivitis was assessed using a modification of the G.I. Loss of attachment was assessed using Ramfjord's technique. Bone loss was diagnosed from standardized bitewing radiographs. Several FORTRAN programs were written to analyze the clinical data.

Preliminary analyses indicate a high prevalence of disease: gingivitis, (71%); attachment loss, (89%); and bone loss, (89%). The average number of sites in the mouth affected with the more advanced form of disease (attachment loss and bone loss) was also high: 32% of the sites had attachment loss, and 22% had bone loss. More detailed analyses are being done, and all research findings will be submitted for publication by Spring 1986. A longitudinal study is planned with the youngest subjects to investigate microbiologic, systemic and other factors that may contribute to the high prevalence of disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00400-01

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Epidemiologic methods for study of periodontitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carlos, James P.	Associate Director for EODP, NIDR	NIDR EODP
Wolfe, Mary D.	Epidemiologist	NIDR EODP
Kingman, Albert	Statistician	NIDR EODP

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Associate Director; Epidemiology Branch

SECTION

Field Studies Section, Biometry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Current methods of summarizing data from epidemiologic studies of periodontal disease (such as the PI, PDI, and CPITN) reduce detailed clinical examination data to one score that summarizes the severity of disease. The purpose of this project is to investigate the possibility of a different summary statistic which will better characterize the clinical data.

The data used for analysis had been collected previously during a longitudinal study of periodontal disease. There were 369 males, age 17-32 years, examined twice, three years apart. The data consisted of 56 attachment loss measurements taken at the mid-buccal and mesio-buccal sites of all teeth excluding the third molars. Criteria were established defining a "diseased" site and a "diseased" subject. Computer programs were written to compute different disease parameters for this population. From these analyses, a bi-variate statistic was derived called the Extent and Severity Index (or ESI), written (E, $\bar{S}$). The ESI summarizes the average extent of sites diseased within the mouth (E) and the average severity of disease in millimeters ($\bar{S}$). Without sacrificing accuracy, it was shown that a half-mouth examination design can be used (mid-buccal and mesio-buccal sites on the second molar through the central incisor, random assignment of two quadrants) which reduces examination time.

E and $\bar{S}$ provide independently useful information, and can be tested with multivariate statistical procedures (such as Hotelling's T^2) to determine significance over time or between groups. The technique and rationale for computing ESI was presented at a conference on periodontal disease, and will be published in the Journal of Clinical Periodontology in 1985-1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00410-01

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Natural History of Periodontal Disease in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

LÖE, HARALD	DIRECTOR, NIDR	EODPP, NIDR
MORRISON, EDITH C.	SENIOR STAFF FELLOW	EODPP, NIDR
SMITH, JACQUELINE I.	STATISTICIAN	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

FIELD STUDIES

INSTITUTE AND LOCATION

NIH, NIDR, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The initiation, rate of progress of periodontal disease and consequent tooth loss and associated microbiologic and immunologic factors are being investigated in a population never exposed to any programs or incidents relative to prevention and treatment of dental disease. Initially 480 male laborers at two tea plantations in Sri Lanka comprised the study group. The study design and baseline findings were reported in 1978. At the initial examination in 1970 the age of the participants ranged between 14 and 31 years. Subsequent examinations occurred in 1971, 1973, 1977, 1982 and 1985. Presently, the longitudinal study covers the age range 14-46 years. During the sixth survey in 1985 there were 161 individuals who had participated in the first survey. The time span between the first and sixth examination was 15 years. A cross-sectional sample was selected from a third plantation in January 1985, and consisted of 169 male tea laborers between 25 and 71 years of age. Three subpopulations have been identified based on interproximal loss of attachment and tooth mortality in both the longitudinal and cross-sectional groups: (1) individuals (8%) with rapid progression of periodontal disease (RP), those (81%) with moderate progression (MP), and a group (11%) who exhibited no progression of periodontal disease beyond gingivitis. The annual rate of destruction in the rapidly progressing (RP) group varied between 0.1 and 1.0 mm., in the moderately progressing (MP) group between 0.05 and 0.5 mm., and in the non-progressing disease groups (NP) between 0.05 and 0.09 mm.

REPORT OF THE MICROBIAL SYSTEMATICS SECTION
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Microbial Systematics Section is charged with establishing a data bank for information describing diverse strains of microorganisms. Special emphasis is placed on the human oral microbiota. For this purpose, collaborative projects are on-going with microbiologists distributed throughout the world.

At present there are tens of thousands of scientists, physicians, public health personnel, and others involved in some aspect of microbiology. The number of microbial strains isolated, characterized, and (in many cases) preserved, by individuals runs into the millions. Hundreds of millions of bits of information have been developed on these strains. However, these data are not resident in a single, centrally located system, permitting rapid and efficient utilization. Because of the large volume of information involved and because, in several applications such as classification and identification, mathematical manipulations of the data are required, electronic processing of these data is necessary.

In collaboration with personnel of the American Type Culture Collection, the Food and Drug Administration (FDA); the Centers for Disease Control (CDC), the Environmental Protection Agency, the Veteran's Administration and numerous academic microbiologists, strain data are being entered into the data bank which provides such services as: data on specific organisms and/or groups of organisms, location of strains with special characteristics, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids for research purposes, aids in quality control of tests, methods, and laboratories, and communication of data via common format.

Data files of orimary data on a large number of microorganisms found in the oral cavity and related types are established. These files provide a resource for asking both ecological and epidemiological questions of interest in dental research.

Programs have been developed and tested to enter, retrieve, and analyze the data in a variety of ways for epidemiological, diagnostic, taxonomic, ecological, etc., uses. The long term goal is to establish a world-wide data network a series of cooperating centers. This goal has been formally adopted with the organization of the Microbial Strain Data Network under the joint sponsorship of two components of the International Council of Scientific Unions: Committee on Data for Science and Technology and World Federation for Culture Collections. As experience grows, improved programs are being designed and implemented to form a computer utility for microbiological data management and analysis. The utility is termed the Microbial Information System (MICRO-IS). The MICRO-IS is in the initial stages of design for adaptation to use in micro- and minicomputer environments. By providing these programs, access to strain data world-wide will be possible under standard conditions.

The system of coding descriptors for computer input of information on bacteria, protozoa, fungi, algae, and its current extension to hybridomas has gained international attention and is of consequence to various components of NIH as well as other agencies in the Public Health Service (specifically FDA and CDC) and the National Science Foundation. The coding system has been adopted as the standard coding method for microbial data by FDA, the World Data Center of the World Federation for Culture Collections, and the Japanese Federation of Culture Collections. The Committee on Data for Science and Technology (CODATA) of the International Council of Scientific Unions has agreed to sponsor the publication of the coding system for world-wide distribution.

CODATA has established a Task Group on a Hybridoma Data Bank for the purpose of establishing an international data bank on hybridoma and monoclonal antibody information. The Task Group is collaborating with the MSS in extending the microbial coding system to hybridomas using the Micro-IS programs. Two staff members of NIH (Dr. M.I. Krichevsky, MSS, NIDR and Dr. B. Janicki, National Institute of Allergy and Infectious Diseases) are members of the Task Group. The staff of the Hybridoma Data Base is located at the American Type Culture Collection. MSS personnel are collaborating closely to provide consultation on systems design and programs to facilitate data transformation and entry. Approximately 4,000 hybridomas have been entered.

Extensive files of descriptions of filamentous and pleomorphic organisms are being assembled. The files cover all the described types of Mycobacteria, blend into the Nocardia, then through the actinomycetes (especially a unique set on oral isolates), and finally, Bacterionema. An extensive cooperative study, covers the oral pleomorphic bacteria (many of which are associated with disease). The study will provide a standard set of well characterized bacteria for the Dental Research community. The data from this study has been incorporated into the files on pleomorphic organisms. These files are being actively analyzed in collaboration with the submitters of the data as well as numerical taxonomists to revise the badly confused taxonomic relationships of these bacteria. Such revision is necessary to avoid the misidentification (leading to erroneous epidemiological conclusions) which are found in some recent dental research literature.

Other files on non-filamentous oral organisms (streptococci, lactobacilli, veillonella, etc.) are being constructed to study correlations among caries activity, phenetic span of characters, serology, source of isolation, and host descriptions.

One of the long term goals in establishing all these files is the establishment of probability tables to allow computer-aided probabilistic identification of oral isolates. Probability matrices, for on-line identification of bacteria (including Gram negative rods, lactobacilli, streptococci, bacilli, etc.) have been constructed. They are available to research workers for use.

COOPERATING UNITS:
V. Jones, Food and Drug Administration
F. Benedict, Food and Drug Administration
R. Gherna, American Type Culture Collection
R. Good, Centers for Disease Control
V. Dowell, Centers for Disease Control
M. Segal, Environmental Protection Agency
L. Wayne, Veterans Administration
V. Sutter, Veterans Administration
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
M. Newman, UCLA
S. Holt, University of Massachusetts
V. Levy-Frebault, Pasteur Institute
H. Sugawara, Institute for Chemical and Physical Research

Algorithms are being developed and tested for aiding in numerical taxonomy of feature by strain matrices too large to be analyzed by existing programs. Both segmentation and heuristic approaches are being investigated.

A program has been developed and is being tested to compare and evaluate methods and/or laboratories when characterizing the same set of strains. The usual statistical packages are not useful because of the predominantly binary (i.e., discontinuous) nature of the data. The algorithm allows comparison of tests or laboratories at the levels of the individual strain (with replicable determinations), species, genus, and overall set for determination of test method equivalences and/or inter-laboratory consistancy.

Computer graphic algorithms are being tested to aid microbiologist in visualizing individual similarities as well as hierarchical group memberships among strains.

Microbial strain data are being entered into a data bank to provide: data on specific organisms, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Data files of primary data on microorganisms found in the oral cavity and related types are established, providing a resource for asking both ecological and epidemiological questions in dental research. Coding conventions have been developed to relate oral clinical parameters with the incidence and distribution patterns of specific microflora. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

Programs are being developed to enter, retrieve, and analyze the data for epidemiological, diagnostic, taxonomic, ecological, uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. The original bacterial system is being expanded to include the algae, yeasts, molds, protozoa, and hybridomas.

Wayne, L. G., Krichevsky, M.I., Portyrata, D., and Jackson, C. K.: Diagnostic probability matrix for identification of slowly growing Mycobacteria in clinical laboratories. J. Clinical Microbiol. 20:722-729, 1984.

Krichevsky, M. I., and Hill, L. R.: Needs and specifications for an international strain data network. United Nations Environmental Program Publications. In Press.

Bussard, A., M.I. Krichevsky, and L.D. Blaine, An International Hybridoma Data Bank: Aims, Structure, Function. In A. J. L. Macario,
E. C. de Macario (ed.), Monoclonal Antibodies Against Bacteria. Academic Press, Inc., Orlando, Florida.

Molitoris, E., M.I. Krichevsky, D.J. Fagerberg, and C.L. Quarles. Effects of dietary chlortetracycline on the antimicrobial resistance of porcine fecal Strepto-coccaceae. Journal of Applied Bacteriology. In Press.

Molitoris, E., M.I. Krichevsky, D.J. Fagerberg, and C.L. Quarles. Effect of dietary chlortetracycline on the antimicrobial resistance of broiler fecal Streptococcaceae. Journal of Applied Bacteriology. In Press.

Molitoris, E., G. McKinley, M.I. Krichevsky, and D.J. Fagerberg. 1985. Comparison of conventional and miniaturized biochemical techniques for identification of animal streptococcal isolates. Microbial Ecology. 11:81-90.

Krichevsky, M.I. Planning and implementation of a computer reporting system. In G.E. Buck (ed.), ASM Manual: Computers in Clinical Microbiology. ASM. In press.

Walczak, C. Computer Software. In G.E. Buck (ed.) ASM Manual: Computers in Clinical Microbiology. ASM. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00044-15 EODP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Handling of Microbial Strain Information by Computers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Krichevsky, Micah I.	Research Chemist	NIDR, MSS
Molitoris, Eric	Microbiologist	NIDR, MSS

COOPERATING UNITS *(if any)*

See attachment

LAB/BRANCH
Epidemiology

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.20	1.50	.70

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microbial strain data are being entered into a data bank to provide: data on specific organisms, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Data files of primary data on microorganisms found in the oral cavity and related types are established, providing a resource for asking both ecological and epidemiological questions in dental research. Coding conventions have been developed to relate oral clinical parameters with the incidence and distribution patterns of specific microflora. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

Programs are being developed to enter, retrieve, and analyze the data for epidemiological, diagnostic, taxonomic, and ecological uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. The original bacterial system is being expanded to include the algae, yeasts, molds, protozoa, and hybridomas.

Genomic diversity as an indicator of stress has been applied to antibiotic resistance in bacteria isolated from animal populations (cattle, swine, chickens). Decrease in diversity is generally explained by non-specific increases in frequency of resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00250-07 EODP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Algorithms for Microbial Systematics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walczak, Cynthia A.	Computer Scientist	NIDR, MSS
Krichevsky, Micah I.	Research Chemist	NIDR, MSS
Mercer, Paula	Computer Programmer	NIDR, MSS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
2.42	2.12	.30

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Algorithms are being developed and tested for aiding in numerical taxonomy of feature by strain matrices too large to be analyzed by existing programs. Both segmentation and heuristic approaches are being investigated.

A program has been developed and is being tested to compare and evaluate methods and/or laboratories when characterinzing the same set of strains. The usual statistical packages are not useful because of the predominantly binary (i.e., discontinuous) nature of the data. The algorithm allows comparison of tests or laboratories at the levels of the individual strain (with replicable determinations), species, genus, and overall set for determination of test method equivalences and/or inter-laboratory consistancy.

Computer graphic algorithms are being tested to aid microbiologist in visualizing individual similarities as well as hierarchical group memberships among strains.

Methods for interconversion between, and interrogation of, equivalent data bases with disparate formats (e.g., controlled vocabulary text versus numeric coding), are being developed as a necessary step in establishing an international Microbial Strain Data Network and for the Hybridoma Data Bank.

Annual Report of the Clinical Trials Section for FY 1985
NATIONAL INSTITUTE OF DENTAL RESEARCH

The prevalence of dental caries in the United States has decreased in the last several years. Further progress toward control of dental caries is likely to come from the identification and development of combinations of anticaries measures that can produce substantial additive effects and that can easily be implemented in wide-scale programs. Clinical trials on the combined use of fluorides and fluorides in combination with sealants continue to receive a major portion of the research effort of the Clinical Trials Section (CTS).

A long-term, longitudinal study to compare the caries-preventive effects of dietary fluoride supplements and weekly fluoride mouthrinsing when each method is used alone, as well as in combination was initiated in September 1981 in Springfield, Ohio, a fluoride-deficient community. The treatment procedures were selected because they are among the most feasible methods of self-administering fluorides and because they are believed to work largely by different mechanisms of action. First follow-up examinations were made in 1983 of participants who were 6-9 years of age. Results for primary teeth showed that subjects in the combination treatment group had 33.2% and 18.9% fewer mean incremental dmfs compared with their cohorts in the rinse and tablet groups, respectively. The observed differences among the treatment groups approached statistical significance, $P = 0.06$. Because of exfoliation, participants at the next follow-up examination in 1986 will be too old (mostly 10 and 11 years of age) to assess caries experience validly in deciduous teeth. Thus, the encouraging two-year results represented the only opportunity to study the effectiveness of the treatments in the deciduous dentition of elementary schoolchildren. For permanent teeth, which were all newly erupted in 1983, incremental caries scores were too small to permit a valid evaluation of effectiveness.

During the past decade, clinical research has demonstrated that dental sealants are highly effective in preventing decay in occlusal surfaces and in buccal pits and lingual grooves of molars. Fluorides, in contrast, are most effective in controlling decay in the smooth surfaces of teeth. When used in combination, sealants and fluorides have the potential of virtually eliminating dental decay. To test this hypothesis, a sealant program was added in 1984 to a program of multiple fluoride administration ongoing in Nelson County, Virginia since 1972. Children in the County's schools ingest daily a fluoride tablet and rinse weekly with a fluoride solution; a fluoride dentifrice is provided for ad libitum use at home. An evaluation of the maximum effectiveness of the combined fluoride program in 1983 showed, for all surfaces, a 65% lower prevalence of dental caries compared with baseline findings. Mesiodistal (smooth) surfaces showed a particularly striking reduction of 90%; essentially all of the remaining decay occurred in pit and fissure tooth surfaces.

Since 1984, sealants have been placed in newly erupted teeth of children in selected grades. Because much skepticism exists about whether sealant placement in school programs can be accomplished cost-effectively, data on the longevity of the sealant material, number of children and sites treated, time required to carry out the procedure, salary of the operators and cost of the materials are being collected.

As part of the design of the study, incipient lesions are being sealed along with the usual sound sites. Although there is a growing body of evidence to show that early lesions once sealed do not progress, many dentists still are reluctant to use pit and fissure sealants for fear of inadvertently covering an incipient lesion. Results of the study may help to establish further the safety of using this proven caries preventive measure.

In 1975, the U.S. Environmental Protection Agency was given responsibility for enforcing drinking water standards, which included establishing a maximum contaminant level for fluoride. Recognizing the need for reliable and current information on the relation between water fluoride concentration and the prevalence of dental fluorosis and dental caries, the CTS conducted an epidemiologic survey in April 1980 of children, ages 8-16, in four areas of Illinois that had naturally-occurring fluoride in their drinking water at concentrations of 1, 2, 3 and 4 times the recommended optimum for their geographic area. Results showed that the prevalence and severity of dental fluorosis were characteristically low in the optimal fluoride area and increased according to the expected dose-response pattern as the water fluoride level increased. Mean caries scores in all three above-optimal fluoride areas were significanlty lower than those in the optimal area, falling to the lowest level at 3 times the optimal fluoride concentration. Early erupting teeth (1st molars and incisors) in children 8-10 years old showed more fluorosis than did the same types of teeth in 13-16 year olds.

Recent anecdotal reports have indicated an increasing prevalence of fluorosis in both fluoridated and nonfluoridated communities. To determine if there have been any secular changes in the prevalence or severity of fluorosis occurring in the Illinois communities, a follow-up survey was conducted in April 1985. The same communities were revisited and, included among the 725 children examined, were 200 children 13-15 years old, who had been 8-10 years of age in 1980. The findings for these older children in 1985 will help determine whether the lower prevalence of fluorosis observed in the older age groups in the 1980 survey resulted from abrasion or remineralization of enamel or from a lower intake of fluoride when these teeth were developing. Additionally, the prevalence and severity of gingivitis was assessed in older children in 1985, to determine whether water-borne fluorides at optimal and above-optimal concentrations influence the periodontal health of schoolchildren.

A protocol for a new study to be conducted by the CTS was approved recently by NIDR's Clinical Research Subpanel. The study is designed to determine the anticaries effect of weekly mouthrinsing in schoolchildren living in an optimally fluoridated and in a nonfluoridated community. In the past, many studies have demonstrated the value of fluoride mouthrinsing in reducing dental decay. Because of the current low prevalence of dental caries, some investigators argue that fluoride mouthrinsing no longer provides clinically significant benefits. Information obtained from the study will help determine if the absolute level of protection from decay is still substantial enough to continue recommending fluoride mouthrinsing as a cost-effective public health measure.

With the reorganization of the National Caries Program and the creation of the Epidemiology and Oral Disease Prevention Program, which has a far broader responsibility for the study and prevention of all oral diseases and conditions, the Program has found itself insufficiently staffed with professional personnel who have expertise in the epidemiology and prevention of periodontal diseases. To help fill this need, Dr. Jukka Ainamo, Professor of Periodontology, Institute of Dentistry, University of Helsinki, Finland, has received an appointment, effective August 5, 1985, as a Visiting Scientist in the NIH Visiting Program. Dr. Ainamo is assigned to the CTS, where his efforts will be concentrated on developing protocols and implementing research for the prevention of periodontal diseases. Of particular relevance is his work in the use of antimicrobials for plaque and gingivitis control. Under Dr. Ainamo's direction, further study of periodontal disease-preventive agents in large-scale, clinical field trials is anticipated.

Contract-supported research activities in FY 1985 included the following:

Encouraging antimicrobial effects of stannous fluoride have been shown in several short-term human studies. An RFP was issued (RFP-NIH-NIDR-5-85-1R) and a contract was awarded to the University of Minnesota, School of Dentistry, to study the effects of routine toothbrushing with a 0.4% stannous fluoride gel on periodontal health. Variables to be measured in this 18-month clinical trial will include the presence of gingivitis, gingival bleeding upon probing, periodontal probing depth and attachment level, supragingival plaque and extrinsic dental stain.

Investigators at the University of Michigan, School of Public Health concluded an epidemiologic survey (RFP No. NIH-NIDR-5-83-2R) to determine the long-term, oral health effects of severe dental fluorosis. Lifelong residents of two communities in New Mexico with optimal (Deming) and more than 4X optimal (Lordsburg) fluoride concentrations in their water supplies received a comprehensive oral health examination. Preliminary findings documented the expected greater prevalence and severity of fluorosis among subjects in Lordsburg. Lordsburg residents also exhibited more occlusal attrition, which the investigators concluded did not have clinical significance. Deming residents, in contrast, had more DMF teeth, were notably more affected by root caries and had fewer arrested caries lesions. Measurements of plaque, calculus and periodontal disease showed no important difference between the two communities. Aside from the cosmetic disadvantages of advanced fluorosis, lifelong exposure to excessive fluorides in a water supply at 4X the optimum concentration for the geographic area did not appear to have any adverse effects on oral health. Results of the survey should be helpful to the EPA in advising the public of the risks of dental fluorosis and in deciding whether a maximum contaminant level for fluoride appropriately belongs in its Primary Drinking Water Regulations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00070-13 EODP

PERIOD COVERED
October 1, 1984 to September 30, 1985 CT 0600045

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Combined self-applied fluorides and sealants for caries prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Heifetz, Stanley B.	Senior Field Investigator	NIDR, DPB, EODP
Meyers, Rhea J.	Clinical Trials Administrator	NIDR, DPB, EODP
Driscoll, William S.	Senior Field Investigator	NIDR, DPB, EODP
Li, Shou-Hua	Statistician (Health)	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS *(if any)*
Nelson County, Virginia Public School System

LAB/BRANCH
Disease Prevention Branch

SECTION
Clinical Trials

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.74	.54	.20

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Baseline dental examinations were conducted in October 1972, of approximately 2200 children (grades 1-12). All participants in grades K-8 chew and ingest daily in school under teacher-supervision a sodium fluoride tablet containing 1 mg F, and rinse weekly with a 0.2% NaF solution. Fluoride-containing dentifrice and toothbrushes are distributed regularly to the children for use at home. Kindergarten classes were invited to participate in the program beginning in the 1976-77 school year. Children in the 7th and 8th grades in Nelson County's junior high school began to participate in the program in the fall of 1978 and 1979, respectively. Beginning in the fall of 1980, high school students in Nelson County began to participate only in the tablet and dentifrice components of the program. For the period covered by this report, children in grades K-12 were participating. In January 1984, a sealant program was added to the ongoing fluoride program. Newly erupted permanent teeth of children in selected grades were sealed with a pit and fissure sealant. The program will run for five years. The combined preventive program has the potential of virtually eliminating dental caries in Nelson County.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00277-06 EODP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dental caries and fluorosis in areas with different water fluoride concentrations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Driscoll, William S.	Senior Field Investigator	NIDR, DPB, EODP
Meyers, Rhea J.	Clinical Trials Administrator	NIDR, DPB, EODP
Heifetz, Stanley B.	Senior Field Investigator	NIDR, DPB, EODP
Kingman, Albert	Statistician (Health)	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention Branch

SECTION

Clinical Trials

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.20	.18	.02

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In April 1980, a cross-sectional survey was made of the prevalence of dental fluorosis and dental caries in seven communities in Illinois that had different concentrations of naturally-occurring fluoride in their public water supplies. The communities were grouped into four categories according to the approximate relation of their actual water-fluoride concentration to the recommended optimal fluoride concentration for the area (1, 2, 3 or 4 times optimal). The study population consisted of 807 school children, ages 8-16, who had lived continuously from birth in their respective communities and had always used the community supply as their primary drinking water source. Dental caries was assessed with the DMFS index and dental fluorosis was measured with Dean's Index and with a newly developed Tooth Surface Index of Fluorosis (TSIF). Fluorosis was assessed independently in each child by each Index. In addition, color photographs of the teeth of some children were taken to depict varying degrees of fluorosis. In April 1982, 316 children from four communities in Iowa with negligible concentrations of fluoride in their drinking water were examined for comparison with the children in Illinois. Two reports of findings based on the Illinois data have been published. One presented basic findings on dental caries and dental fluorosis as measured with Dean's Index; the other presented findings based on the new Tooth Surface Index of Fluorosis. A third report, presenting the Iowa data along with supplementary findings from Illinois, is currently being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00310-05 EODP

PERIOD COVERED
October 1, 1984 to September 30, 1985 CT 0600144

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*. Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Heifetz, Stanley B.	Senior Field Investigator	NIDR, DPB, EODP
Meyers, Rhea J.	Clinical Trials Administrator	NIDR, DPB, EODP
Li, Shou-Hua	Statistician (Health)	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS *(if any)*
Springfield, Ohio, Public and Non-Public Schools

LAB/BRANCH
Disease Prevention Branch

SECTION
Clinical Trials

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.63	.51	.12

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Approximately 1700 kindergarten and first grade children were randomly assigned to one of the following groups:
Group I - Rinses once every week in school with a 0.2% sodium fluoride solution.
Group III - Ingests once a day in school a sodium fluoride tablet containing 1 mg of fluoride.
Group II - Carries out the regimens for both Group I and Group III.

The method of assignment resulted in three comparable groups, each containing about 560 children. Participants carry out their assigned treatments in classrooms under the supervision of a teacher. Treatments will be administered for a minimum of eight and nine years for children initially in the first grade and kindergarten, respectively. Baseline dental examinations were conducted in September 1981. The prescribed treatments were initiated shortly after the examinations were completed.

In September 1984, the fourth school year of treatments began in the classrooms. First follow-up dental examinations were made in October 1983 of 1,154 participants who were then in grades 2 and 3. Supplies have been ordered, schedules arranged and local personnel identified for the start-up of the fifth school year of treatments in September 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00396-01 EODP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Caries, fluorosis, gingivitis and calculus in areas with different water-fluoride levels.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Driscoll, William S.	Senior Field Investigator	NIDR, DPB, EODP
Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Meyers, Rhea	Clinical Trials Administrator	NIDR, DPB, EODP
Heifetz, Stanley B.	Senior Field Investigator	NIDR, DPB, EODP
Kingman, Albert	Statistician (Health)	NIDR, EB, EODP
Morrison, Edith	Senior Staff Fellow	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention Branch

SECTION
Clinical Trials

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.74	.51	.23

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is a followup to a previous survey carried out by the NIDR in April 1980 in four areas of Illinois that have fluorides naturally occurring in their drinking water supplies at concentrations of approximately one, two, three and four times that recommended as optimal for those areas. The same communities were revisited to examine children in two of the same age groups (8-10 and 13-15 years) that were evaluated in 1980. Study participants in the 8-to-10 year age group comprised all children of those ages who received parental consent to participate and whose residence histories indicated that they had resided continuously since birth in their respective communities. The 13-to-15 year old group comprised only those children who were examined as 8-to-10 year olds in 1980. Examinations were conducted in April 1985 to assess the prevalence of dental caries and dental fluorosis in both age groups. In addition, the prevalence of gingivitis and dental calculus was assessed in all children in the 13-to-15 year age group.

ANNUAL REPORT OF LABORATORY METHODS SECTION

NATIONAL INSTITUTE OF DENTAL RESEARCH

The intraoral fluoride releasing-device (IFRD) developed by Southern Research Institute for the National Caries Program continues to be of significant interest. Devices with different fluoride release rates are being used to determine dose-response effects of controlled-release fluoride and the residual effects of this type of device are being investigated by comparing treatment periods of different lengths. Results of the studies repeatedly indicate a beneficial anticaries action which appears to exist for a significant time.

In a clinical trial of the IFRD, subgingival plaque was monitored for S. mutans and viable bacteria in twenty patients during pretreatment, treatment and posttreatment phases. All the microbiologic parameters examined showed a significant increase during the treatment phase compared to baseline. The percentage of S. mutans and the microbial index scores had returned to pretreatment levels when inspected four weeks after detaching the devices; however, the total CFU remained elevated.

Dicalcium diphosphate (DCPD) has been shown to increase the uptake of fluoride in dental enamel. Use of this agent with an IFRD has shown significant incorporation of fluoride into the enamel of rat molars. DCPD in conjunction with SnF_2 has also been tested in a human population for antiplaque activity. The SnF_2 treatments restricted plaque extent and pH drop in plaque following a sucrose rinse. The DCPD pretreatments augmented fluoride uptake by enamel without interfering with the antiplaque effects of the SnF_2 treatments. Whether the increased uptake of fluoride will increase protection against caries is presently being investigated.

Efforts continue to establish a collection of microorganisms considered to be associated with periodontal diseases. This includes attempts to obtain specific bacteria that have been reportedly used as antigens for development of antisera. The methodology for production of antisera to many of the bacteria is being developed and/or refined to meet our particular needs for intramural research as well as for use in human epidemiologic studies. Because many of the potential pathogens in periodontal diseases require special handling (e.g. reduced media, atmosphere, etc.) and storage, considerable effort has been devoted to this activity.

The cariogenic potential of foods commonly consumed as "snacks" continues to receive considerable attention. There exists lack of agreement among researchers as to methodology that will provide a dependably replicable assessment. During the past year we have modified existing methods and incorporated them into a regimen which we feel will be acceptable to most researchers, be logistically uncomplicated and be replicable. Five foodstuffs commonly eaten as "snack" foods have been tested using the regimen. Preliminary results appear to suggest the Program Model could be adopted for a standardized method to screen various foodstuffs for potential cariogenicity.

An investigation of antisera effect on glucose uptake by S. mutans 6715 shows that antisera to both whole cells and components known to be present on the cell surface reduced glucose uptake relative to normal serum. Reduction levels generally ranged from 25-40% of the maximum level which occurred after about 5 minutes of incubation in the presence of labeled glucose.

An epidemiologic investigation involving approximately 1200 12-14 year old children to investigate possible protection against dental caries and hardness of water (with and without fluoride) was performed during the year. Preliminary results indicate the (1)the fluoride effect was obvious and obscured any effect from hardness; and, (2) if there was a "hardness" effect it was probably small and not detected with the population sizes.

Our Program is involved in Phase 1 clinical trials of new and/or existing antiplaque, anticaries and antigingivitis agents. The populations used most frequently in these trials are 18-26 year-old individuals. There exists a need to establish in a "normal" population the occurrence of soft tissue lesions (e.g. aphthous ulcers), the week to week variation in tooth staining and the observation over time of gingival bleeding. A short longitudinal collaborative study has begun with Georgetown University to assess these parameters.

The section has devoted considerable time to assessing non-sucrose sweetening agents and dental caries in rats. Various regimens and supplemental diets have been investigated and many have been adopted for the purpose of refining our animal model system.

Recent advances in high performance liquid chromatography (HPLC) column technology have made it possible to rapidly analyze protein samples using HPLC. Using and modifying this technology we have shown that it compares favorably with the radial immunodiffusion (RID) and rocket immunoelectrophoresis (IEP) procedures in accuracy and precision. It is possible that this approach can be used for detection of substances introduced into the mouth during antimicrobial therapy.

Increasing concern over the relationship between fluorosis and water fluoride concentration, fluoride in toothpastes/mouthwashes and fluoride in the food chain has led to our reevaluation of some microanalytic methods for plaque fluoride analysis. Samples of dental plaque from monkeys exhibited similar levels of fluoride by heat mediated and silane mediated diffusion methods. However, the fluoride appeared to be unevenly distributed in dental plaque and varied in amount from week to week.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00112 12 EODPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preclinical screening of anticaries agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Adderly, Donna D.	Chemist	EODPP LMS NIDR
Bartkiewicz, Andrea	Chemist	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Little, Wayne A.	Microbiologist	EODPP LMS NIDR
Mirth, Dale B.	Research Chemist	EODPP LMS NIDR
Monell-Torrens, E.	Bio Lab Technician	EODPP LMS NIDR

COOPERATING UNITS *(if any)*

Clinical Investigations and Patient Care Branch, NIDR (Michael W. Roberts) American Dental Association Health Found. National Bureau of Standards, Gaithersburg, Md. (L.C. Chow)

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.36	0.64

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal objectives of this project are part of the ongoing effort identifying antiplaque and anticaries agents suitable for short-term clinical investigation, as well as to develop methods for assessing the clinical potential for these agents.

The present study investigated the effects of DCPD treatments on the following: (a) uptake of fluoride by molars; (b) caries incidence; and, (c) pH levels in dental plaque of rats wearing an intraoral fluoride releasing device (IFRD). The DCPD treatments significantly enhanced enamel uptake of fluoride released by the IFRD. Rats wearing an IFRD exhibited a low caries incidence with or without a DCPD pretreatment. pH responses among groups were similar.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00234 08 EODPP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Develop method of intraoral telemetry of various ions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Stiles, Horace M.	Chief, LM Section	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Kingman, Albert	Statistician	EODPP BS NIDR

COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Branch (T.L. Talbot, H.W. Tipton)
Commissioned Officers Dental Clinic (A.D. Guckes)

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.40	0.19	0.40

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory is using both intraoral and extraoral methods of measuring the pH of dental plaque. This response is one of several used for screening food and therapeutics that might promote oral health. The present study tested the feasibility of using antimony pH electrodes for use in measuring the effects of SnF2 and detected the expected pH inhibition in the SnF2 treated groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00281 06 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of oral fluids using high performance liquid chromatography (HPLC)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mirth, Dale B.	Research Chemist	EODPP DPB NIDR
Adderly, Donna D.	Chemist	EODPP DPB NIDR
Bartkiewicz, Andrea	Chemist	EODPP DPB NIDR
Li, Shou-H.	Visiting Statistician	EODPP EB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 0.45	PROFESSIONAL: 0.04	OTHER: 0.41

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent advances in high performance liquid chromatography (HPLC) column technology have made it possible to rapidly analyze protein samples using HPLC. The purpose of the present project was to evaluate high performance liquid chromatography as a means of analyzing biological fluids such as saliva and plaque fluid and to see if it was feasible to use HPLC to quantitate natural or added substances in saliva.

The results suggest that HPLC can be used to quantitate natural constituents of saliva such as s-IgA with accuracy and precision comparable to other commonly used methods such as radial immunodiffusion. HPLC should also be useful for monitoring the salivary levels of antibacterial agents after a person uses a mouthrinse or dentifrice containing an antibacterial agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00282 06 EODPP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of an intraoral fluoride-releasing device in rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mirth, Dale B.	Research Chemist	EODPP DPB NIDR
Adderly, Donna D.	Chemist	EODPP DPB NIDR
Bartkiewicz, Andrea	Chemist	EODPP DPB NIDR
Monell-Torrens, Esteban	Bio Lab Tech. (Animal)	EODPP DPB NIDR
Li, Shou-Hua	Visiting Scientist	EODPP BS NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*
Southern Research Institute, Birmingham, AL 36255

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.76	0.06	0.70

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous in vitro and in vivo studies have shown that an intraoral fluoride-releasing device developed by the Southern Research Institute for the National Institute of Dental Research will deliver fluoride at a steady rate for up to six months. This project will evaluate the anticaries effect and mechanism of action of the fluoride-releasing device in rats. Results have shown that rats that had an intraoral fluoride-releasing device in their mouth developed significantly fewer caries on all surfaces than untreated or placebo treated animals. The data indicate that the marked caries reduction produced by the fluoride-releasing device was due to topical effects of fluoride. Additional data suggest that the cariostatic effects of the intraoral fluoride-releasing device may persist for a significant time period after the device is removed from the mouth; therefore, intermittent, as well as continual use of an intraoral fluoride-releasing device may provide significant protection from dental caries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00319 05 EODPP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A simple microdiffusion technique for measuring fluoride in biological samples

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Shern, Roald J.	Principal Investigator	EODPP DPB NIDR
Kennedy, John B.	Biologist	EODPP DPB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 0.61	PROFESSIONAL: 0.10	OTHER: 0.51

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously this laboratory reported that various microanalytic methods detected different levels of fluoride in aliquots of plasma from the same sample. Currently we are assessing the effects of DCPD pretreatments on fluoride uptake by human dental plaque following SnF2 treatments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00371 03 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of antisera on glucose uptake by streptococcus mutans 6715

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Little, Wayne A.	Microbiologist	EODPP DPB NIDR
Ciardi, Joseph E.	Research Chemist	MS LMI NIDR
Alexander, Catherine R.	Clerk Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

University of Oslo, Norway (Prof. Gunnar Rolla)

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.12	0.01	0.11

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study was to investigate the hypothesis that a protective mechanism of salivary antibody may be via interference with the glucose uptake system of acidogenic bacteria such as S. mutans. The effect of antisera to whole cells or cell wall components on glucose uptake by S. mutans 6715 was examined. Stationary phase 6715 cells were treated with test serum and incubated at 37 degrees C in the presence of 14-C -glucose. Samples were removed at timed intervals and measured in a liquid scintillation counter for radio-active uptake. Antisera to both whole cells and components known to be present on the surface of the cells reduced glucose uptake relative to normal serum.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00390 02 EODPP

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cariogenicity of palatinit in rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Brunelle, Janet	Chief B Section	EODPP DPB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Lee, James	Computer Technician	EODPP EB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.46	0.10	0.36

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Palatinit is a non-sucrose sweetener which is reported to be aproximately half the sweetness of sucrose. Because of its potential use a a sucrose substitute by individual and industrial consumers, its cariogenic potential should be determined.

The experiment consisted of four groups of rats (10/group) which were fed one of the following diets: Group 1(Suc) -NIH Diet 2000 (56 percent sucrose); Group 2(RCS) -NIH Diet 2114 (56 percent raw corn starch); Group 3(P1) -25 percent palatinit plus 31 percent raw corn starch; Group 4(P2) -56 percent raw corn starch replaced by 56 percent palatinit). During the first week all the animals consuming the 56 percent palatinit developed diarrhea and died leaving three groups for the remainder of the study. The experiment was performed using a Konig-Hofer progammabale feeding machine which delivered 17 meals/17 hour period of the assigned diets. An additional 12 meals of diet 2114 were provided all four groups as a supplement. All animals were initially infected with S. mutans 6715. Oral infection levels were determined twice weekly. After 56 experimental days the rats were sacrificed.

Resultant mean smooth surface caries scores (± S.E.) were: Group 1 (56 percent sucrose) - 9.5(1.9); Group 2 (56 percent raw corn starch) - 4.9(1.18); and Group 3 (palatinit 25 percent, raw corn starch 31 percent) - 3.4(1.04). It would appear that palatinit at a concentration that would not incite gastric problems is a potential substitute for sucrose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00401 01 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microbiological parameters associated with an intraoral fluoride releading device

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Little, Wayne A.	Microbiologist	EODPP DPB NIDR
Shern, Roald J.	Laboratory Scientist	EODPP DPB NIDR
Mirth, Dale B.	Research Chemist	EODPP DPB NIDR
Alexander, Catherine R.	Clerk Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

University of Maryland Dental School, Baltimore, Maryland

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 0.91	PROFESSIONAL: 0.20	OTHER: 0.71

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Twenty adolescents, fitted with intraoral fluoride releasing devices (IFRD), were monitored over a six month period to determine if there were certain microbiological changes in the plaque at the attachment site of the IFRD. Increases in the percentage of S. mutans and total cultivable flora were observed in supragingival plaque after placement of the IFRD. There was also an increase in the proportions of motile rods and spirochetes in the subgingival plaque.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00404 01 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cariogenicity of Lylose

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Lee, James	Computer Technician	EODPP EB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.14	0.21

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lylose is a sweetening agent developed in the U.K. by Tate and Lyle PLC. It is approximately 0.4 the sweetness of sucrose. Because of its potential use as a sucrose substitute by individual and industrial consumers, its cariogenic potential should be determined. Initially it was compared with two concentrations of sucrose using the rat animal model and a programmed feeding regimen.

Four groups (10/group) of rats were fed the following diets: Group 1 -Diet NIH 2000 (56 percent sucrose); Group 2 -Diet NIH 2114 (56 percent raw corn starch); Group 3 -Basic diet NIH 2000 with Lylose (56 percent) replacing sucrose; and Group 4 -Basic diet NIH 2000 containing 28 percent sucrose and 28 percent raw corn starch. The experiment was performed using a Konig-Hofer progammabale feeding machine which delivered 17 meals/17 hour period of the assigned diets. An additional 12 meals of diet 2114 was provided all four groups as a supplement. All animals were initially infected with S. mutans 6715. Oral infection levels were determined twice weekly. After 56 experimental days the rats were sacrificed.

Considering caries scores for smooth surfaces only, those animals eating lylose had scores lower than the animals consuming 56 and 28 percent sucrose (p 0.03 and 0.01 respectively). Therefore, lylose may be considered a potential substitute for sucrose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE00405 01 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Replicate screening of foodstuffs for potential cariogenicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Kingman, Albert	Statistician	EODPP EB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Lee, James	Computer Technician	EODPP BS NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.29	0.71

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A programmed feeding regimen was used to feed rats a cariogenic diet or one of five foodstuffs commonly consumed as "snack foods". A nutritionally adequate diet was presented as supplemental feedings to assure sufficient nutritonal elements not supplied by the foodstuffs alone. Each foodstuff was replicated at least one time. The objective was to evaluate this regimen for wide scale use in the assessment of potential cariogenicity of various foods when compared to one of high cariogenicity.

Preliminary results appear to support the use of this method. Results of caries scores, weight gain and food consumption are presently being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00406 01 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Epidemiology of dental caries and water hardness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Rodgers, Patricia	Statistical Assistant	EODPP EB NIDR
Davis, Patricia	Statistical Assistant	EODPP EB NIDR
Gregg, Mary	Statistical Assistnat	EODPP EB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.08	0.32	0.76

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This was considered a pilot cross-sectional study to investigate a potential activity between protection against dental caries and water hardness with and without fluoride. Preliminary results are as follows:

	N	Water Hardness*	Fluoride(ppm)	DMFS
Group 1	519	Less than 50	Less than 0.2	5.2
Group 2	316	Approx. 250	Less than 0.2	5.1
Group 3	182	Less than 50	0.8	2.7
Group 4	146	Greater than 250	1.0	2.8

These results indicate that 1) the fluoride effect was obvious and obscured any effect from hardness; and, 2) if there was a '"hardness" effect it was probably small and not detected with the population sizes. A future study will concentrate on high and low hardness only.

*as Calcium carbonate mgms/liter

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE00407 01 EODPP

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Soft tissue lesions, tooth color variation and gingival bleeding in a population

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Lee, James	Computer Technician	EODPP EB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*
Georgetown University Dental School, Washington, DC

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 0.53	PROFESSIONAL: 0.21	OTHER: 0.32

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phase 1 clinical trials frequently involve 18-26 year-old individuals. Subjects are monitored frequently for the occurrence of soft tissue lesions (e.g. aphthous lesions), tooth staining and other manifestations in the oral cavity However, there is a paucity of data about these occurrences in a "normal" population, i.e. without any treatment or intervention.

Fifty otherwise healthy 18-26 year-old students at Georgetown University are examined six times over the period of one year. At each examination a)any soft tissue lesions are noted and photographed; b)teeth are photographed; and c)a bleeding index determined.

At the completion of the study data will reflect changes in these parameters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00408 01 EODPP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Short-term clinical trials of antiplaque agents N01-DE-52484 CT 0600075

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Shern, Roald J.	Principal Investigator	EODPP DPB NIDR
Kennedy, John B.	Biologist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

ADA Health Foundation, Research Unit, NBS, Gaithersburg, MD 20899
School of Dentistry, College of Medicine, National Taiwan University, Taipei

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.31	.25	.06

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are: (1) to identify, adapt and pretest methods of measuring the bacterial and chemical composition of dental plaque and saliva; and, (2) to conduct short-term clinical studies of agents which might be capable of restricting dental plaque and caries.

The present studies investigated the effects of DCPD pretreatments used weekly as a tray applied gel and SnF_2 used twice daily as an oral rinse on dental plaque and gingivitis and fluoride uptake by the outer enamel. Stannous fluoride treatments restricted plaque extent and the pH drop in plaque following a sucrose rinse. DCPD pretreatments augmented fluoride uptake by the enamel without interfering with the antiplaque effects of the SnF_2 treatments.

INTRAMURAL RESEARCH PROGRAM

ANNUAL REPORT OF THE CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH
1984-1985

The Clinical Investigations and Patient Care Branch functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinically oriented research programs; (2) to render clinical care to specified patients of the Clinical Center; (3) to offer consultation on oral and dental problems to other institutes; (4) to encourage and to provide support, consultation and facilities for clinical research activities of other Branches and Laboratories within the Institute; and (5) to sponsor an oral medicine training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research oriented dental clinicians.

The past year has seen a considerable solidification of laboratory research programs with a clear focus on two major areas: glandular epithelial cell transport processes and regulatory aspects of N-linked protein glycosylation. Likewise a strong center of clinical research has been built first around the dry mouth/xerostomia program and second, around diagnostic and management problems posed by specific compromised patient groups (oncology, congenital disorders). Collaborative interactions with other laboratories or branches, both within and outside NIDR, have increased substantially. There is an extremely high level of effort, cooperation, flexibility and understanding by Branch personnel which has allowed us to make great strides towards reaching our overall goals.

Patient Care Section

The Patient Care Section conducts the daily operation of the NIDR Dental Clinic and is the focus of clinical oral and dental health concerns at NIH. The Section provides a wide range of diagnostic consultative services to NIH clinical care and research programs. Staff dentists and dental hygienists routinely participate in medical rounds and patient care discussions thus integrating oral health care concerns to total patient management.

The Section's staff are deeply involved in the continued development of the Dental Staff Fellow program. The Section is primarily responsible for the clinical training, and introduction to clinical research, of Dental Staff Fellows. Scheduled rounds, a Fellowship lecture series and a journal review are conducted weekly throughout the year. The lecture series brought in speakers from outside the NIH as well as from the various Laboratories and Branches of the Intramural Research Program, NIDR.

This past year has seen a major effort, by Patient Care Section dentists and hygienists, to develop a cohesive clinical research program based on the unique patient problems seen at NIH. This effort has been tangibly aided by the close working relationship with the Clinical Investigations Section, as well as by collaborations with other branches at NIH. Two major protocol based study areas are beginning to develop. The first addresses management problems of patients under going cytotoxic chemotherapy. Because of studies in the NCI, this group forms a large percentage of Section patients. Thus we are in a unique position to be able to evaluate different regimens of oral health care in a rigorous, controlled fashion. The second general area involves patients with congenital disorders; particular attention being directed towards the characterization of dental-craniofacial manifestations. Included in these studies are patients with osteogenesis imperfecta, Albright's hereditary osteodystrophy, Reiger's syndrome, familial Sotos syndrome, and precocious puberty.

The Patient Care Section has also continued its academic affiliations with the Baltimore College of Dental Surgery of the University of Maryland, Georgetown University School of Dentistry, and Montgomery College. These arrangements provide graduate dental students, senior dental hygiene, dental assistant, and dental laboratory students an opportunity to experience alternative practice settings beyond those offered in the school core curriculum, as well as provide our staff with academic clinical dental teaching responsibilities. The Section also participates in the NIH Clinical Electives Program, and this past year one senior dental student served in an eight week program which emphasized providing dental care to medically compromised patients in a hospital environment.

Clinical Investigations Section

The general theme of this section's investigative efforts is understanding the regulation of membrane-associated, epithelial cell physiological processes. The model tissues studied are mammalian salivary glands and the focus of study includes (1) understanding trans-acinar cell transport (serosa to mucosa) of fluid and electrolytes; (2) characterizing receptor-regulated mechanisms involved in secretory protein N-glycosylation; and (3) defining the etiology of, and developing treatments for, specific salivary gland secretory dysfunctional states and associated oral disorders.

Considerable effort is directed at understanding biochemical mechanisms involved in the formation of saliva. It is well accepted that saliva has a critical role in the defense, and functional maintenance, of all oral tissues. Saliva contains water and electrolytes, derived from serum, and specific exocrine proteins synthesized by glandular epithelial cells. Salivary glands are useful models of exocrine secretory processes and studies with these glands have proved valuable to our understanding of basic concepts of secretion as well as to appreciating pathogenesis in conditions such as cystic fibrosis. At present we primarily utilize rat salivary glands with both *in vitro* and *in vivo* systems.

One main thrust of study is directed towards understanding secretory events related to water and electrolyte movement. These responses are primarily under $_1$-adrenergic and muscarinic-cholinergic control. Because the autonomic receptors elicit their effects in great part due to changes in membrane permeability, we have directed considerable effort at clarifying ion fluxes associated with early events in secretion.

For example, using intact rat parotid cells, _in vitro_, we have been able to in detail investigate the role of Na^+ in Ca^{2+} mobilization elicited by adrenergic stimulation. We have followed up initial observations made last year by careful studies which clearly show that a Na^+ out > Na^+ in gradient is not requisite for stimulated Ca^{2+} fluxes in these cells. Furthermore we have shown that the Ca^{2+} efflux associated with the Na^+ ionophore monensin, a finding previously reported by many laboratories, is (1) not kinetically reflective of adrenergic agonist Ca^{2+} mobilization and (2) is not blocked by two classes of Ca^{2+} antagonists which inhibit the adrenergic response. Other studies with intact parotid cells have suggested the presence of a Na^+, K^+, $2Cl^-$ co-transport mechanism. We have shown Cl^- transport is diminished (1) by the loop diuretics furosemide and bumetanide and (2) by replacement of Na^+ or K^+ in the incubation media. These results reflect major characteristics of the Na^+, K^+, $2Cl^-$transporter. Movement of Cl^- is also affected by carbachol, a situation expected if this transporter were to be important in driving fluid and electrolyte secretion.

Last year we reported our success at being able to obtain functional basolateral membrane vesicles suitable for direct transport study. During this year we have focused our attention on mechanisms responsible for control of Ca^{2+} extrusion from cells (and thus maintaining low levels of cytosolic Ca^{2+}, ie nM range). Our studies have shown that events which raise cellular levels of cyclic AMP increase ATP-dependent Ca^{2+} transport. These include -adrenoreceptor stimulation, non-receptor activation of A. cyclase with the diterpene forskolin and exposure of cells to an analog of cyclic AMP, 8Br cAMP. This alteration in Ca^{2+} pump activity can be kinetically characterized by an increased Km as well as an increased Vmax. In effect, it would appear that the parotid gland responds to adrenergic secretory stimuli by resetting its Ca^{2+} handling mechanisms to apparently allow for sustained secretion. This effect is specific, since it is not induced by a muscarinic-cholinergic agonist, shows a dose-dependent response to a -adrenoreceptor agonist and is prevented by exposure to a -antagonist. We also have utilized these membrane vesicle preparations to further probe Ca^{2+} handling mechanisms using the aging paradigm, described in past reports. Interestingly while parotid basolateral membranes from young adult and aged rats show no differences in Na^+/Ca^{2+} exchange, a consistent reduction in ATP-dependent Ca^{2+} transport (~25-50%) is seen in vesicles from membranes of aged rats.

In addition to secreting water and electrolytes, parotid acinar cells secrete the majority (>80%) of parotid salivary proteins. During the past year we have shown that rat parotid cells respond to vasoactive intestinal polypeptide (VIP) by increased exocrine protein secretion (eg. amylase) through a cyclic AMP mediated mechanism. The general VIP response is analagous to -adrenergic responses and indicates that parasympathetic stimulation results in both a "cyclic AMP" (thru VIP) and a "Ca^{2+} mobilizing" (thru acetylcholine) response, similar to sympathetic stimulation (which accomplishes both responses with norepinephrine).

We have also continued our studies on -adrenoreceptor control of protein N-glycosylation. Consistent with our observation that the -adrenergic agonist isoproterenol elicits a 2-3 fold increase in synthesis of mannosylphosphoryldolichol and oligosaccharide-PP-dolocol in parotid cells, we observed that such treatment also increases (~50%) the activities of 4 microsomal, dolichol-linked glycosyltransferases. We explored the mechanism of activation of one such enzyme, Man-P-Dol synthase, in detail and discovered that we could mimick the intact cell response to -adrenergic agonists, by exposing microsomal membranes to cyclic AMP-dependent protein kinase _in vitro_. The kinetic characteristics of activated enzyme for the two types of treatment were identical; no change in the Km for GDP-mannose, but a 50% activation of Vmax. We have also obtained evidence that -adrenoreceptor stimulation accelerates (~2 fold) the processing (from high mannose to complex forms) of N-linked oligosaccharrides in a high molecular weight parotid secretory glycoprotein (220Kd). During this reporting period we also have shown that cyclic AMP induced upregulation of N-linked glycosylation is not limited to rat parotid glands. Both a normal human diploid fibroblast strain and a permanent line of Chinese hamster ovary cells exhibit such responses in culture. Also microsomal membranes from bovine brain and hen oviduct, after treatment with cyclic AMP-dependent protein kinase, show significantly enhanced (~50-60%) Man-P-Dol synthase activity.

We have also made some progress in our ability to grow _in vitro_, important cellular elements associated with saliva formation. First, we have been able to culture, and characterize, glandular (from adrenal tissue) endothelial cells. Endothelial cells provide a barrier through which fluid and electrolytes destined to enter the primary saliva, must pass. In addition many systemic hormonal factors known to affect salivary gland function, must cross the endothelial cells prior to reaching the salivary target cells. We have also begun studying membrane transport properties of these cells. Secondly we have been able to obtain cultures of rat submandibular duct cells and transform them to an apparently permanent line with methylcholanthrene. These cells possess a Na^+-dependent amino acid transport system (system A). Using the model amino acids, -aminoisobutyrate and methylaminoisobutyrate, system A transport was shown to be pH and temperature dependent, as well as kinetically comparable in the two cell populations.

The focus of this section's human clinical research is understanding the etiology and oral sequelae of conditions which result in salivary gland dysfunction. Central to this activity is the dry mouth/xerostomia study which has involved over 150 patients. We have made considerable progress in standardizing diagnostic approaches to patients complaining of xerostomia. In patients that present with diminished or absent resting saliva output, but are capable of stimulated secretion, we have found that 5 mg of pilocarpine, p.o., increased salivary flow for 2-3 hours and afforded patients considerable subjective relief. This double-blind crossover study gave impetus to a second phase of study, now ongoing, with timed release pilocarpine capsules (also 5 mg), taken by patients at home for 30 days.

The population available in the xerostomia study also has allowed us to investigate other, "salivary-related", oral physiological functions. We have extended our previous observations on gustatory performance by persons with grossly impaired salivation. Nine individuals, in this category, have been found who are essentially free of subjective taste complaints and display normal suprathreshold gustatory function. This supports our earlier contention that saliva is not critical to maintain the functional integrity of the gustatory apparatus. Some of these patients however do show alterations in performance at threshold concentrations of tastants. This supports the notion that thresholds reflect an aspect of sensory function unrelated to daily life tasks.

We have also devoted considerable effort to studying the oral phase of swallowing utilizing ultrasonic imaging methods. Many dry mouth patients displayed alterations in tongue movement both during dry swallows or swallows with a water bolus present in the mouth. The latter observation attests to the important role of salivary constituents (vs only water), in permitting a normal swallow through its presumed lubricatory properties. In parallel we've begun to establish normal characteristics of the oral phase of swallowing across the life span. Thus far we have observed considerable lengthening of the time necessary to complete a "swallowing cycle" (ie from initiation to hyoid elevation) in older persons. This timing increase however is still considerably smaller than temporal changes seen in patients with frank neurologic disease.

Most important, we have been able to successfully translate several questions raised in patients, to laboratory research models. For example, many individuals require chronic use of drugs affecting -adrenoreceptor function. Our laboratory studies have suggested an important role of -adrenoreceptors in regulating N-protein glycosylation (see above). Accordingly we have begun studying an in vivo rat model of chronic -adrenoreceptor agonist and antagonist therapy. We assessed the ability of salivary proteins, from the various animal study groups, to aggregate oral bacteria and mediate adsorption of bacteria onto hydroxyapatite. We observed significant alterations in these functions in saliva from drug treatment groups compared to that seen with control secretions. We have also continued our studies on phenothiazine-induced inhibition of salivary secretion by testing an hypothesis we suggested last year. We proposed that phenothiazine-induced salivary alterations involve a competitive inhibition of neurotransmitter binding to muscainic-cholinergic receptors. Our studies this year have been entirely consistent with this notion. Chlorpromazine inhibits radioliand ([^{3}H]-QNB) binding to receptors rapidly (< 1min) with an IC$_{50}$ of $\sim 10^{-7}$M. At 10^{-5}M chlorpromazine is essentially as effective as equimolar amounts of the muscarinic antagonist atropine.

The unique mission of the Clinical Investigations and Patient Care Branch in the NIDR is made possible by the blending of the academic-, clinical problem oriented-Patient Care Section together with the strongly basic science oriented, yet clinical problem appreciating, Clinical Investigations Section. We have made considerable progress since the reorganization of the Branch in 1982. We anticipate continued forward movement in our efforts to address questions of importance to the understanding and management of oral diseases.

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CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

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Wright, W.E., Haller, J.M., Harlow, S.A. and Pizzo, P.A. An oral disease prevention program for patients receiving radiation and chemotherapy. Journal of the American Dental Association. 110(1): 43-47, 1985

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-09 CI

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Taste and Its Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Weiffenbach, J.M.	Research Psychologist	NIDR CIPC
Baum, B.J.	Clin Dir/Chf Clin I	NIDR CIPC
Fox, P.C.	Dental Officer	NIDR CIPC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to elucidate the mechanisms by which oral sensory and perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity and pleasantness for taste stimuli at higher, more commonly encountered levels of strength. These methods, applied to the study of possible age-associated changes have provided insights into basic mechanisms of chemosensory perception. Functional variation under pathologic circumstances are now being measured. Currently objective evaluations are being made of oral sensory disturbances occurring in association with systemic disease, salivary gland dysfunction or as an isolated complaint. Assessments of olfaction and of oral tactual sensitivity are obtained when they can contribute to an understanding of oral sensory function in relation to the complex stimuli encountered in everyday life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00332-04 CI

PERIOD COVERED
October 1, 1984-September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberts, Michael W.	Chief Patient Care	NIDR	CIPC
Brahim, Jaime S.	Senior Staff Fellow	NIDR	CIPC
Drum, Margaret A.	Clinical Staff Dentist	NIDR	CIPC
Folio, John	Senior Staff Dentist	NIDR	CIPC
Freeman, Ronald D.	Dental Staff Fellow	NIDR	CIPC
Haller, Julie M.	Dental Hygienist	NIDR	CIPC
Harlow, Shelley A.	Dental Hygienist	NIDR	CIPC

COOPERATING UNITS *(if any)*
Laboratory of Pathology and Pediatric Branch, NCI; Clinical Epidemiology Branch, NCI; Inter-Institute Genetics Program, CC; Ophthalmic Genetics and Pediatric Branch, NEI; Arthritis & Rheumatism Br.,NIADDK; Georgetown University

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.15	2.50	0.65

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews Chart reviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical case studies of unusual interest and clinically related research are being conducted on a variety of dentally related subjects. Research techniques being utilized include chart and literature reviews and evaluation of various therapeutic regimens.

PHS 6040 (Rev. 1/84) GPO 904-917

PROFESSIONAL PERSONNEL

Roberts, Michael W.	Chief Patient Care	NIDR	CIPC
Brahim, Jaime S.	Senior Staff Fellow	NIDR	CIPC
Drum, Margaret A.	Clinical Staff Dentist	NIDR	CIPC
Folio, John	Senior Staff Dentist	NIDR	CIPC
Freeman, Ronald D.	Dental Staff Fellow	NIDR	CIPC
Haller, Julie M.	Dental Hygienist	NIDR	CIPC
Harlow, Shelley A.	Dental Hygienist	NIDR	CIPC
Martin, Sue E.	Pathologist	NIDR	CIPC
Macynski, Alice A.	Clinical Nurse (General)	NIDR	CIPC
Perna, John J.	Dental Staff Fellow	NIDR	CIPC
Troullos, Emanuel S.	Dental Staff Fellow	NIDR	CIPC
van der Ven, Peter F.	Dental Staff Fellow	NIDR	CIPC
Van Story-Lewis, Patricia	Dental Staff Fellow	NIDR	CIPC
Wright, William E.	Senior Dental Staff	NIDR	CIPC
Bale, Allen E.	Medical Staff Fellow	NCI	CE
Parry, Dilys M.	Geneticist	NCI	CE
Mulvihill, John J.	Chief Clinical Genetics Section	NCI	CE
Kaiser-Kupfer, Muriel I.	Chief Ophthalmic Genetics	NEI	CB
Guckes, Albert D.	Chief CODC	CC	NIH
Pizzo, Philip A.	Chief Pediatric Branch	NCI	PB
Broring, Charles L.	Chairman Pedodontics	Georgetown Univ.	
Magyarosy, E.	Visiting Fellow	NCI	LP
Termine, John J.	Ch. Mineralized Tissue Resbr	NIDR	MTR
Klippel, John H.	Senior Investigator	NIADDK	AR
Chu, E.W.	Chief, Cytopathology Section	NCI	LP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00336-04 CI

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Salivary gland secretory mechanisms during normal and altered functional states

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Baum, B.J.	Clin Dir/Chf Clin. I	NIDR CIPC
Ambudkar, I.	Visiting Associate	NIDR CIPC
Brown, A.	Guest Researcher	NIDR CIPC
Helman, Y.	Visiting Fellow	NIDR CIPC
Kawaguchi, M.	Visiting Fellow	NIDR CIPC
Scott, J.	Visiting Associate	NIDR CIPC
Turner, J.	Visiting Scientist	NIDR CIPC

COOPERATING UNITS *(if any)*
LCMB, NIA

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.55	0.65

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize both in vitro and in vivo preparations of rat salivary glands primarily to understand mechanisms controlling saliva formation. We have focused our studies on autonomic neurotransmitter regulation of secretory events in normal, aging and two types of therapy-induced salivary gland dysfunctions (X-radiation, phenothiazines). During the present reporting period specific areas of study were (1) further examination of mechanistic processes in Ca^{2+} handling by parotid basolateral plasma membrane; (2) determination of the presence, and importance, of a Na^+, K^+, 2 Cl^- transport mechanism in parotid fluid secretion; (3) the possible regulatory role of VIP (vasoactive intestinal polypeptide) in parotid secretion; (4) the evaluation of chlorpromazine as a competitive muscarinic antagonist; and (5) the development and characterization of an epithelial cell line from rat submandibular glands.

List of Professional Personnel continued

van der Ven, P.	Dental Staff Fellow	NIDR CIPC
Roth, G.S.	Research Chemist	NIA LCMB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00337-04 CI

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Physiological Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fox, P.C.,	Dental Officer	NIDR CIPC
Baum, B.J.	Clin Dir/Chf Clin I	NIDR CIPC
van der Ven, P.	Dental Staff Fellow	NIDR CIPC
Weiffenbach, J.	Research Psychologist	NIDR CIPC
Martin, S.E.	Pathologist	NIDR CIPC
Sonies, B.C.	Speech Pathologist	CC RM
Hathorn, J.	Medical Staff Fellow	NCI POB
Gahl, W.	Medical Officer	NIADDK HGB

COOPERATING UNITS *(if any)*
RM, CC; DR, CC; POB, CC; HGB, NICHD; Columbia University; SUNY, Buffalo; University of Florida

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.0	1.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the function of various oral tissues during physiologic aging and in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness) utilizing the inpatient and outpatient services of the Dry Mouth Evaluation Clinic. Specific diagnostic approaches have been developed to aid in establishing the etiology of salivary gland dysfunction and defining criteria necessary for management decisions. A treatment protocol for selected patients with demonstrable functional gland mass yet inadequate basal salivary performance continues, employing a regimen of oral administration of the parasympathomimetic drug, pilocarpine. In addition, detailed studies of salivary-associated oral complaints (eg taste and oro-pharyngeal swallowing disorders) have continued. Such studies include evaluation of oral sensorimotor performance across the adult life-span in order to better understand dysfunctional states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00338-04 CI

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Oxygen Radicals in Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hoffeld, J.T.	Dental Officer	NIDR CIPC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies were conducted to determine the species-specific and differentiation stage-specific nature of three activities of inflammatory cells: proliferation, disulfide-cleaving capacity and expression of gamma-glutamyltranspeptidase (gGTP). Earlier studies indicated that the latter two activities create a reducing (antioxidative) environment around the cells, thereby being permissive of the proliferation. Multiple monocyte/macrophage/myeloid cell lines of either human or murine origin were cultured under various specified conditions so that direct comparisons under identical conditions could be made. The question of influence of differentiation state was only partially answered by the selected array of cell lines; more-differentiated cells proliferate less vigorously; disulfide-cleaving activity and gGTP expression were, in all cases proportional to proliferation, as with the more rapidly dividing cells. The species differences (and, thereby, the applicability to human use) were quite clearly defined: 1) for proliferation, cells of equal differentiation state were equal, irrespective of species; 2) human gGTP was greater than the murine gGTP; and 3) human disulfide-cleaving activity was greater or equal to the murine activity. These findings further contribute to the utility of manipulation of these cell activities in the potential control of inflammatory cell activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00361-03 CI

PERIOD COVERED

October 1, 1984-September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Application of Immunocytochemistry to the Diagnosis of Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin, Sue E.	Pathologist	NIDR	CIPC
Moshiri, S.	Visiting Fellow	NCI	LP

COOPERATING UNITS *(if any)*

Cytopathology Section, Laboratory of Pathology, NCI, NIH

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The diagnosis of malignant cells in body fluids and fine needle aspirates can be extremely difficult using morphological criteria alone. Using lymphoid cell surface markers we have developed a technique for the immunocytochemical diagnosis of lymphoma in cytological specimens. This methodology is applicable to all types of malignant disease, provided one has suitable cell surface markers. We have investigated various markers for their usefulness in the diagnosis of metastatic carcinoma in body cavity fluids and needle aspirates. This work should greatly enhance the power of cytological diagnosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00362-03 CI

PERIOD COVERED
October 1, 1984-September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dental Development in Patients with Precocious Puberty

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberts, Michael W.	Chief Patient Care	NIDR	CIPC
Li, Shou Hua	Statistician (Health)	NIDR	EB
Comite, Florence	Clinical Associate	NICHHD	DEB
Pescovitz, Ora H.	Medical Staff Fellow	NICHHD	DEB
Cutler, Gordon B., Jr.	Chief Developmental Endocrinology	NICHHD	DEB
Loriaux, Lynn D.	Clinical Director	NICHHD	DEB
Hench, Karen B.	Associate Investigator	NICHHD	CC

COOPERATING UNITS *(if any)*
Developmental Endocrinology Branch, NICHHD, NIH

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Attempts are being made to determine if there are any correlations between skeletal age, chronological age, tooth and dental root formation in patients with precocious puberty. Oral examinations have been completed on 145 children and panographic radiographs obtained on 130. Using the most accurate available dental growth and development standards comparative studies are being made. These studies will involve both cross sectional and longitudinal evaluations. The NICHHD is a cooperating unit in the study and they have assigned their project number Z01 HD 00610.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00372-03 CI

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

N-Linked Protein Glycosylation and β-Adrenoreceptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kousvelari, E.	Senior Staff Fellow	NIDR CIPC
Baum, B.J.	Clin Dir/Chf Clin I	NIDR CIPC
Banerjee, D.	Visiting Associate	NIDR CIPC
Ciardi, J.E.	Research Chemist	NIDR LMI

COOPERATING UNITS (if any)

LMI, NIDR; M.D. Anderson Hospital; Univ. Texas - San Antonio

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clincial Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Saliva contains a broad spectrum of proteins which are known to play a pivotal role in maintaining the integrity of the hard and soft oral tissues. Many of the proteins secreted from the parotid gland are glycoproteins and carry N-linked oligosacchrides, so-called N-linked glycoproteins. To study the mechanisms involved in processing, synthesis and secretion of N-linked secretory glycoproteins, we have utilized in vitro cell and microsomal membrane preparations from rat parotid glands. We have shown that β-adrenergic receptor stimulation increases [^{3}H]-mannose incorporation into newly synthesized glycoproteins by a cyclic AMP mediated mechanism. During the present reporting period we have 1) further characterized the processing of oligosacchrides after β-adrenoreceptor stimulation and demonstrated the effect of a glucosidase I, II inhibitor, deoxnejorimycin, on the maturation and secretion of parotid glycoproteins; 2) isolated fractions enriched in the four secretory glycoproteins (17,32,38 and 220 Kd) exhibiting glycosylation changes after β-adrenoreceptor stimulation and produced a polyclonal antiserum which reacts with all four; 3) observed increased activity of several dolichol-linked glycosyltransferases after β-adrenoreceptor stimulation and demonstrated that for one enzyme, Man-P-Dol synthase, a cyclic AMP-dependent protein kinase phosphorylation in vitro results in a kinetically identical activation as seen with β-adrenoreceptor stimulation in the intact cell; and 4) initiated investigations in vivo on the effects of β-adrenergic receptor stimulation on functional properties of N-linked secretory glycoproteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DE-00395-01 CI

PERIOD COVERED
October 1, 1984- September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Study of a Dentin Bonding Agent and Composite Resin in Posterior Teeth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberts, Michael W.	Chief Patient Care	NIDR CIPC
Folio, John	Senior Staff Dentist	NIDR CIPC
Guckes, Albert D.	Chief CODC	CC NIH
Moffa, Joseph P.	Chief Clinical Research Branch	Letterman Army Institute of Research

COOPERATING UNITS *(if any)*
Clinical Research Branch, U.S. Army Institute of Dental Research, Letterman Army Institute of Research

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will evaluate the clinical performance of a small particle bimodally filled hybrid proprietary composite resin and a dentin bonding system. This composite resin system will be employed for the restoration of class II carious lesions in posterior teeth. Its performance will be compared to that of a conventional amalgam. Fifty restorations of each of the two materials will constitute the study. Each restoration will be evaluated for clinical performance using a standardized method. Also, an attempt will be made to quantify any loss of material due to surface disintegration or wear.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00398-01 CI

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endothelium as an Active Functional Barrier in Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Banerjee, D.K.	Visiting Associate	NIDR CIPC
Ornberg, R.L.	Senior Staff Fellow	NIADDK LCBG
Pollard, H.B.	Medical Officer	NIADDK LCBG

COOPERATING UNITS *(if any)*

LCBG, NIADDK

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To understand the hormonal regulation of saliva production, it is necessary to investigate how these chemical mediators cross the endothelial barrier from the systemic circulation. As a probe to study such an event, capillary endothelial cells have been isolated and established into a stable cell line. These cells grow in a relatively simple media, form specialized intercellular connections and eventually generate macroscopic capillary-like structures. The ready availability of such cells, capable of capillary formation in vitro in the absence of added factors, constitute a simplification for cell biological and biochemical studies and provide a model system where capillary proliferation is thought to play a central role in pathophysiology.

ANNUAL REPORT OF THE DIAGNOSTIC SYSTEMS BRANCH

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Diagnostic Systems Branch (DSB) is concerned with the isolation and identification of factors limiting performance obtainable from predominantly noninvasive diagnostic systems. Particular emphasis is directed toward development of image-based systems designed primarily for dentistry, but the scope is broad enough to include research applicable to a variety of biomedical tasks.

In keeping with DSB's multidisciplinary approach to systems optimization, existing resources are deployed preferentially in collaboration with other agencies having common interests.

Current activities continue to emphasize two long-standing projects:

1) Development and Evaluation of Improved Diagnostic Systems

2) Enhancement of Diagnostic Images.

These represent extensions of a precedented commitment to design and produce a clinically useful, computer-based x-ray system having primary use and application in dentistry. A third project reported last year focused on the use of new noninvasive methods to improve the detectability of small changes in the shape of the mandibular condyle in order to correlate joint damage with observed neuromuscular and occlusal patterns.

This latter work initially emphasized the application of an existing algorithm to analyze computerized tomographic (CT) slices in order to determine factors limiting the accuracy of estimates of joint destruction. The basic idea was to produce a detailed three-dimensional replica of joint constituents from serial CT slices. The accuracy of this method for description of bony joint constituents was determined to be adequate for certain diagnostic applications but it was found to be relatively insensitive to significant changes in capsular morphology. For this reason CT based methods were supplemented with a newer technology better suited to the functional description of soft tissues; i.e. magnetic resonance imaging (MRI).

Hence, the third project described last year has been expanded in context and scope to include the exploration of MRI as well as CT based methods as applied to a much larger variety of potential diagnostic applications. The decision to broaden the base of this research also stems from the fact that recent changes in the spectrum of visiting research personnel within DSB have resulted in a much more balanced approach to the investigation of diagnotic systems.

Hence, this expanded project may be described more appropriately as "Exploration and Assessment of New Diagnostic Modalities", and has been so renamed this year to reflect this more general emphasis.

System-development research is an extension of that reported in previous years wherein a prototype miniaturized tomosynthetic x-ray system is being fabricated with extramural support, in collaboration with private interests and the National Bureau of Standards. The device is unique in that it contains no moving parts and is capable of producing a video image in near-real time which can be fed back to a computerized x-ray generator to make possible task-specific control of exposure. Such a system has many advantages which facilitate reliable detection and spatial location of tiny changes in the teeth and supporting bone, occurring over relatively long periods.

Specific accomplishments in this area include solidification of technical design specifications and the establishment of a variety of subcontracts to specialized manufacturers as the first step in acquisition of custom-designed hardware essential to the fabrication of a working prototype.

These activities represent a significant deviation from plans reported last year based on collaboration with Ridge Inc. The collaboration with this commercial producer of industrial x-ray equipment led to the design and fabrication of a special intraoral dental x-ray tube suitable for digital image subtraction and tomosynthetic reconstruction. Subsequent tests of the prototype were so promising that Ridge, Inc. agreed to seek venture capital to develop the entire system for commercial applications in industry as well as in medicine and dentistry.

As a consequence of this activity early in FY '85, the entire company was purchased by IRT, Inc. which rendered all pending agreements made by Ridge subject to review by the parent company. This delayed indefinitely plans for further development and negated existing obligations on the part of Ridge management to provide development beneficial to dental applications. Consequently, alternative plans were activated which circumvented the need for a cost-intensive intraoral source and led to an extension of the existing interagency agreement between NIDR and the Radiation Physics Group at the National Bureau of Standards. This contract solidifies design options appropriate for dental applications and renders prototype development feasible without proprietary concerns complicating support mechanisms.

The new system is better suited to dental applications by virtue of the fact that the scanning source now will be located outside the oral cavity which minimizes proximity distortions and obviates the need to shield the tongue from the primary x-ray beam. It also simplifies design and hence is potentially less expensive to produce than the Ridge-sponsored alternative described above.

Like the Ridge intraoral tube, the new design also facilitates the potential for synthesis of any desired x-ray projection from the radiographic information acquired during a single automated scanning sequence.

Preliminary data confirm that image subtractions produced from appropriately weighted projection data are compromised less by differences in projection geometry than comparable data produced using nearest-neighbor, control projections. In theory, this implies that it is now possible to relate radiographic data obtained with the new prototype system with data obtained from the wealth of retrospectively produced periapical radiographs already existing in patient files. Also, as reported last year, the relatively high speed and disparity control afforded by this new filmless design are still preserved, and the need for fabricating custom stints for each different x-ray projection, likewise, has been eliminated.

As now specified, the system is comprised of an extraoral source of x rays having an extended target on which the focal spot can be scanned continuously under computer control. This will be aimed toward a raster-scanned high-speed intra-oral x-ray detector which will produce real-time video signals to be stored in solid-state memory. Resulting x-ray projections can be visualized instantaneously and also transferred to magnetic tape for off-line processing by existing resources both within and outside DSB.

Subtraction of images obtained from such a digital data base requires the development of new software to implement the necessary visual superposition of appropriate radiographic projections.

As a first step toward this development DSB programming staff have undertaken the simulation of a hard-wired digital-image rotator. This involves the creation and testing of a lookup table capable of transferring a conventional raster-based digital display into one characterized by polar coordinates. Preliminary results confirm that such an approach produces reproducible rotations having known stable truncation artifacts. Such an approach assures that image distortion produced by digital rotation can be controlled in ways that are independent of the number of times the rotation process is repeated.

This work also anticipates results reported last year dealing with automated image registration and analysis. Of particular importance in this regard are the delineation of appropriate task-specific measures of image similarity. Recent investigations have confirmed preliminary observations reported last year, and have been extended to demonstrate for the first time that similarity measurements can be used to estimate the angular disparity of an arbitrary projection by comparing it with a series of related projections having known geometric properties.

Computer programs for estimating the size of nodular defects from radiographic images likewise have been refined significantly over the last twelve months. The latest version uses a closed polygon derived from radially directed gradient measurements to approximate the lesion border. By comparing the contrast exhibited by the lesion relative to its background in histogram-compensated images, correlations of better than 0.95 have been made with actual nodular defects induced and measured in vitro.

Related research also has extended methods reported earlier for processing x-ray images differing in contrast and dynamic range. The latest nonparametric algorithms are both simpler and faster as well as considerably more robust than their parametric counterparts described last year. Objective tests of both methods have been performed and demonstrate conclusively a significant reduction in contrast differences between images corrected using the new algorithm.

Work continues on the development of methods for eliminating unwanted blur associated with tomosynthetic methods of image reconstruction. Specifically studied are task-specific bases for system optimization. By eliminating all spatial frequency components lower than those compatible with the correlation distance inherent in the projection geometry, computer measurements derived from actual radiographs confirm the theory that all remaining details in reconstructed slices are truly independent of the structures known to lie in other slices. Conversely, extension of the idealized iterative reconstruction scheme described last year to include information from more remote slices has shown that the extended algorithm is likewise insensitive to uncorrelated noise, and further, the image contrast can be improved significantly over previous efforts.

Concurrent efforts by other researchers were directed at the determination of associated risks as measured dosimetrically. The basic idea was to confirm theoretical predictions that radiation exposure attributable to the new system would be reduced over the status quo in spite of the need for eight radiographic projections of each region of diagnostic interest. For this work cadaver specimens were used to assure realistic approximation of soft tissue as well as hard tissue attenuation and scatter patterns produced by the prototype system. The resulting data demonstrate conclusively that tomosynthetically produced images suitable for a variety of diagnostic applications can be produced with reduced exposures for the following reasons:

1) The quantum yield of the proposed system is significantly

higher than that associated with nonscreen film,

2) Conventional dental radiographic images are not quantum-limited in the sense that contrast rather than noise determines the detectability of small signals,

and

3) The signal-to-noise ratio produced by tomosynthetic reconstruction from eight component projections is determined by the composite exposure rather than that associated with each projection individually.

Given that the new system will permit task-specific bases for optimization of exposure, and that ideally such tasks should be limited by quantum statistics, some effort has been extended toward optimization of noise-specific image-filtration schemes. High on the priority list this year has been the development of computationally efficient techniques for median filtering of radiographic data to eliminate some of the quantum fluctuations intrinsic to low-dose systems. To this end, an algorithm has been created which makes efficient use of commercially available image-storage hardware (solid-state memory frame-buffers), to perform two-dimensional median filtration of stored images in near real time.

Another aspect of system development presumes that diagnostic performance obtainable radiographically can be improved by the use of multiple x-ray projections of each region. This assumption is being tested rigorously in a controlled fashion through a comprehensive investigation of the diagnostic influence of exposure geometry on the detectability of induced lesions in human teeth. Preliminary data confirm that angle-dependent sampling variance has a profound effect on the radiographic detectability of interproximal defects which extends beyond that attributable to the obvious overlapping of dental contours associated with oblique projection geometries.

A new aspect of our approach to diagnostic systems reported last year for the first time involves the role played by a priori information in the maximum performance obtainable from existing sources of diagnostic data. Of particular concern is the prevalence of the underlying disease processes and its impact on system performance expressed in terms of task sensitivity and specificity. A collaborative effort is underway with Dr. Samuel Zeichner of the radiological staff at the University of Pittsburgh to do a blind evaluation of an existing radiographic data base to determine the degree to which panoramic vs. full-mouth periapical radiographic surveys are useful independently in detecting biopsy-confirmed, intraosseous, nodular lesions of the jaws. Preliminary data suggest that statistically significant differences between these two modalities can be anticipated with reasonable assurance and further that the time required for radiographic interpretation as well as a variety of measures of diagnostic accuracy are likely candidates for meaningful comparison. Extensive efforts to estimate the prevalence of these disease processes are also underway and preliminary data obtained from several state-wide sources suggest that most of the serious disorders tabulated are so rare as to place in question the rationale that their occult presence justifies routine prophylactic radiographic examination.

A related study being done in collaboration with physicians at Children's Hospital National Medical Center involves the formal development of a logistic model to predict bases for early discharge of patients undergoing intensive care in an effort to optimize the deployment of limited resources and better contain associated costs. The basic idea is to use Bayesian statistics to predict mortality risks from physiologic measurements obtained in the ICU. By developing a new continuous variable to characterize the severity of disease based on these physiologic measurements, it has been possible to formulate a logistic regression model which has been tested on an independent source of ICU data. The resulting prediction accuracy afforded by the model has been found to be significantly improved over the status quo. This model has since been applied to data from eight other hospitals distributed nationwide and shown to be institution independent. Hence, it provides a superior method for determining the discharge from or acceptance to the ICU of individual patients when compared to the respective methods now in use at these institutions.

In recent years considerable effort has been expended in the transfer of technology developed earlier by DSB. Perhaps the most widely spread example involves the use of a digital method to compare periapical radiographs obtained from patients suffering from periodontal disease who are undergoing a variety of controlled treatment regimens. The technique involves registration of successively exposed films using a computer-based projection system and subsequent subtraction of stored images after correcting for contrast differences attributable to incidental changes in the x-ray exposure and processing chemistry. Findings based on data obtained from controlled periodontal investigations within the last year suggest that these methods are capable of not only improving the detectability of osseous lesions but providing objective means for visualizing and quantifying both the lytic processes and the associated host responses. To this end DMS investigators are currently looking into alternative diagnostic methods to provide independent means for monitoring such activity. These include site-specific measures of oxygen tension using a tiny fiberoptic probe developed recently by scientists in the Biomedical Engineering and Instrumentation Branch, NIH, and the use of a new cadmium telluride semiconductor probe to measure nuclear medicine activity in periodontal bone, developed by investigators at Harvard University.

Other new diagnostic modalities being explored this year include magnetic-resonance imaging (MRI) of dental tissues and the use of colorimetric optical systems to detect the presence of oxygenated blood in the pulp chamber of vital teeth. As reported last year, MRI has produced striking displays of intracapsular structures in the temporomandibular joint with the aid of new surface coil technology. As a consequence, this research has been expanded to include a collaborative investigation with colleagues in Diagnostic Radiology and Harvard University. This controlled animal study compares MRI with an established predictor of periodontal bone destruction, i.e. the uptake of a diphosphinate tagged by a radiopharmaceutical as measured using the cadmium telluride probe described above. Also to be monitored are induced gingival inflammatory changes, both chronic and acute, and salivary gland activity. Preliminary data show that MRI can reliably track salivary responses having time constants measured in minutes.

In support of these activities DMS has implemented significant staffing changes and has extended its collaboration with other research facilities. These now include:

The State University of New York in Buffalo
The University of Michigan
Forsyth Dental Research Center in Boston
Harvard School of Dental Medicine
The University of Minnesota
The University of Pittsburgh
The University of Pennsylvania
The National Bureau of Standards
School of Dentistry, Goteborg, Sweden
School of Medicine, Linkoping, Sweden
The University at Groningen, The Netherlands
The Free University at Amsterdam, The Netherlands
The Children's Hospital National Medical Center in Washington, D.C.

This year marks the end of Dr. David Roberts' two-year term as a Visiting Scientist with DSB. Dr. Roberts was instrumental in extending diagnostic applications research into the field of CT based morphometrics and MRI. He is planning to continue work on his NIDR initiated projects at the University of Pennsylvania. Dr. Roberts is being replaced by Dr. Samuel Zeichner from the University of Pittsburgh. Dr. Zeichner is a dental radiologist with research interests in nuclear medicine and MRI as well as being a DMS collaborator in the evaluation of diagnostic performance obtainable from existing radiographic modalities.

Our senior systems programmer, Ms. Ann Davis, recently accepted a much more lucrative position with a high-technology, commercial systems engineering group. Immediate compensatory plans involve contracting for applications programming essential to development of the prototype x-ray system through an existing interagency agreement with NBS. This permits remaining DMS programming staff to shift from predominantly applications projects toward essential systems-based responsibilities formerly held by Ms. Davis. Such a strategy assures increased program flexibility by freeing up a tenured position but is considered tentative pending an actual test of the long-term workability of this arrangement.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00065-14 DS

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development and Evaluation of Improved Diagnostic Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Webber, Richard L.	Chief Diagnostic Sy	NIDR	DS
Ruttimann, Urs E.	Senior Staff Fellow	NIDR	DS
van der Stelt, Paul F.	Visiting Fellow	NIDR	DS

COOPERATING UNITS (if any)

Childrens' Hospital National Medical Center

Radiation Physics Group, National Bureau of Standards

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.49	1.40	1.09

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is an extension of previous work directed toward the study of noninvasive methods to determine spatial and temporal relationships existing between tissues of clinical interest. The approach involves in vitro modelling of promising systems and the development of prototypes suitable for clinical evaluation.

Recent work has focused on studies directed toward development of a versatile computerized radiographic system designed to be used both fluoroscopically and off-line to produce images which can be subtracted to show small changes in tissue occuring over long intervals of time, and combined in ways permitting tomographic display of specific slices of individual teeth.

An all-electronic prototype is being fabricated which couples a specially developed extraoral x-ray source having a tiny focal spot which can be positioned under computer control on an extended target, to a miniaturized video imaging system. This design differs from earlier prototypes in that it is both simpler and better suited to dental applications.

This system has been simulated using images produced with components having comparable performance specifications. Results confirm that clinically meaningful subtraction images can be produced without the need for an occlusal stint to stabilize projection geometry and further that the system can be used to synthesize any desired x-ray projection from a series of related projections having known geometric properties.

Other diagnostic systems are being studied which involve the predictability of a new measure of illness severity as applied within the context of a hospital intensive care environment.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00211-09 DS

PERIOD COVERED
October 1, 1985 - September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Enhancement and Processing of Diagnostic Images

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Webber, Richard L.	Chief Diagnostic Sy	NIDR	DS
Ruttimann, Urs E.	Senior Staff Fellow	NIDR	DS
van der Stelt, Paul F.	Visiting Fellow	NIDR	DS

COOPERATING UNITS (if any)
Radiation Physics Group, National Bureau of Standards

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.83	1.50	1.33

CHECK APPROPRIATE BOX(ES)
xx (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is an extension of previous work involving the creation, development and testing of image processing techniques designed to improve diagnostic performance.

Current work has centered on methods relevant to the processes of radiologic image subtraction and tomosynthesis. Work continues in the area of automated manipulation to facilitate registration. Particular emphasis has been placed on 1) methods for correcting contrast differences existing between serially obtained radiographic image projections, 2) methods for eliminating noise produced by quantum fluctuations in radiographs, 3) methods for quantifying the apparent size of lesions from associated diagnostic images and 4) methods for precisely manipulating digital images in ways essential for registration preparatory for image subtraction.

A new nonparametric algorithm for correcting differences in radiographic contrast has been developed which is simpler, faster, and more robust than its parametric counterpart described last year. Another computer program has been developed which permits quantum fluctuations intrinsic to low-dose radiographic systems to be eliminated by means of rapid median filtration using available frame-buffer hardware. Improved measures of image similarity and techniques for quantifying the size of lesions from subtracted radiographs facilitate unbiased image analysis essential to quantitative radiology within the constraints of a clinically practical system. The latter makes use of a lesion perimeter detection scheme based on radially determined gradient measurements abstracted from the subtraction image. By comparing contrast exhibited by the lesion relative to its background in histogram-compensated images, correlations of better than 0.95 have been made with nodular defects of known size.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00373-03 DS

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Exploration and Assessment of New Diagnostic Modalities

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Webber, Richard L.	Chief Diagnostic Sy	NIDR	DS
Roberts, David	Visiting Scientist	NIDR	DS

COOPERATING UNITS (if any) Harvard School of Dental Medicine, Boston, MA
Radiology Associates, Medical Imaging Section, University of PA
Diagnostic Radiology Department, Clinical Center, NIH
Biological Engineering and Instrumentation Branch, NIH

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.50	1.17	0.33

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects   xx (b) Human tissues   ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with the evaluation of a variety of new and existing diagnostic techniques which have yet to be applied to biomedical diagnostic applications having particular relevance to dentistry. It is an extension of a more restricted project described last year which dealt with the development of quantitative methods for measuring factors believed to be associated with the myofacial pain dysfunction (MFPD) syndrome. Methods studied include photoplastic recording of strains associated with occlusal forces, computerized tomographic (CT) analysis of changes in size and shape of tissues of diagnostic interest, nuclear-medicine based techniques for tracking bone remodelling, and magnetic resonance imaging (MRI) as a noninvasive indicator of physiologic and pathologic changes in oral-facial tissues.

Extensive measurements of occlusal stress patterns in patients known to be suffering from MFPD have yet to be analyzed statistically. Confirmatory data from CT analysis of cadaver specimens show that the maximum error in computed vs. actually measured volumes of the mandibular condyle is less than five percent with nominal values closer to one percent. Associated soft tissues such as the capsule of the temporal mandibular joint have been noninvasively displayed clearly for the first time with MRI. Preliminary findings using this modality also suggest that salivary gland activity may be monitored dynamically with no recognized biological risk or other side effects.

Another new diagnostic modality being explored this year for the first time involves the use of a filtered optical system to colorimetrically detect the presence of oxygenated blood in the pulp chamber of vital teeth. Preliminary findings based on an analysis of red colored beads in the pulp chamber of extracted teeth appear promising.

ANNUAL REPORT OF THE LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

NATIONAL INSTITUTE OF DENTAL RESEARCH

Marked changes have occurred during the last year in personnel and in program direction. Dr. Gary Grotendorst, who had developed a major research initiative in LDBA in wound healing research, left in the fall of 1984 to take a position at the Medical University of South Carolina. The main aims of our research in this area will shift to the molecular events in repair. Also Dr. Victor Terranova who had developed an active program on cancer cell metastasis moved to a position in the Revlon Biotechnology Research Center in Rockville. Work will continue at LDBA in this area but with more emphasis on molecular mechanisms. Several new people have joined the laboratory including Dr. Laura Raimondi (pharmacologist), Dr. Jeannette Graf (pediatrician), Dr. Adriana Albini (cell biologist), Dr. Makoto Sasaki (orthopedic surgeon), Dr. Ornella Selmin (biochemist), and Dr. Lyman Smith (orthopedic surgeon).

Two organizational changes have been recommended at the suggestion of the Scientific Councilors. These include the creation of a Cell Biology Section and a Molecular Biology Unit, two areas where LDBA scientists are at the forefront of research. There is a rapid increase in the application of molecular biology to all areas of research.

RESEARCH ACCOMPLISHMENTS

Studies on the Structure and Regulation of Connective Tissue Genes

Laminin - Laminin is a large protein discovered in LDBA which is specific to basement membrane. It has a variety of biological activities particularly on neurons, epithelial cells, and differentiating cells. The laminin molecule has 3 distinct chains; A(Mr=400,000), B1(Mr=210,000) and B2(Mr=200,000). Recombinant DNA clones have been prepared against these chains. The most progress has been made with the B1 chain. Here some 90% of the chain struture has been deduced from its nucleotide sequence (Fig. 1). The data suggest that there are 4 major domains. Two domains at the amino terminal end form large globules which are visualized also by electron microscopy. Synthetic peptides from portions of the molecule have been prepared based on the sequence data. These will be used to prepare antibodies and to test for biological activities.

Type IV Collagen Chains - The murine α1(IV) collagen cDNA has been cloned and partially sequenced. Genomic clones covering more than 50 kilobases of this gene have been isolated. Some 7 exons from the helical region of the chain have been sequenced. These show that this chain has a very different gene structure from that of the interstitial collagens indicating separate evolution of this collagen.

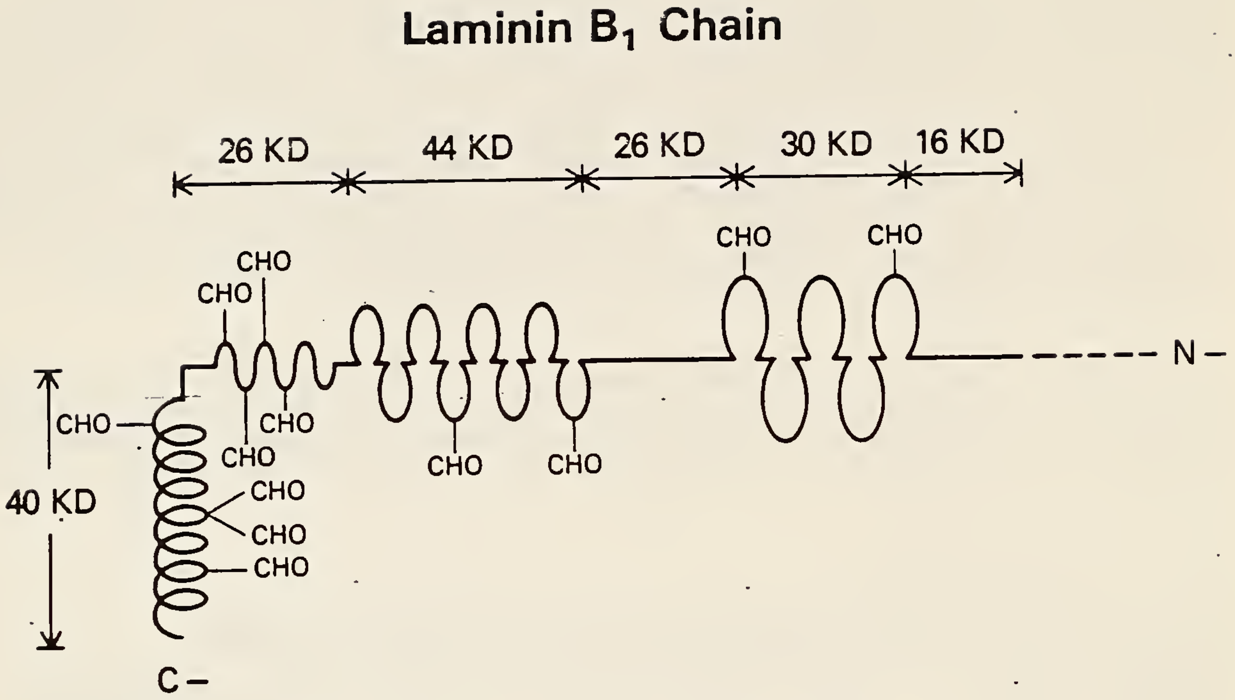
Laminin B$_1$ Chain

Figure 1: Schematic model of the B1 chain of laminin. The structure was deduced from the cDNA sequences.

Link Protein - Link protein is a small (MW = 43,000) glycoprotein which stablizes the interaction of cartilage proteoglycan with hyaluronic acid and allows the formation of super molecular aggregates. cDNA clones to link protein have been isolated and the 800 bp cDNA was sequenced and found to contain two homologous stretches of 19 amino acids. These regions show strong homology to the reported amino acid sequence from the hyaluronic acid binding region of cartilage proteoglycan, suggesting a common structure for these domains in link protein and proteoglycan.

Type II Collagen - Type II collagen is the major extracellular protein synthesized in chondrocytes. To study regulation of the type II collagen gene, we have isolated the gene for the rat type II collagen and characterized the promoter region of this gene. We have constructed a series of plasmids which contain various lengths of the flanking sequence of the type II collagen gene and the bacterial gene for chloramphenical acetyltransferase (CAT). We introduced these plasmids into chick chondrocytes and analysed for CAT activity. The preliminary results suggest that a sequence upstream from nucleotide -380 is necessary for the efficient transcription of the type II collagen gene.

The use of genomic and cDNA clones for specific connective tissue components in murine transgenic experiments is planned. Both viral infection and direct injection modes of gene transfer will be attempted using collagen type II promotor coupled to marker genes to determine if tissue specific action can be produced.

Developmental Biology and Pathological States - The availability of well characterized murine cDNA probes has made it possible to study the steady-state levels of mRNA for these basement membrane constituents. To date, we have examined the appearance of specific mRNAs in embryonic mouse tissues and followed changes in their levels with age. These studies have provided valuable baseline data for the investigation of diseases such as diabetes mellitus.

We have isolated and characterized genomic clones for approximately 20 Kb of the human α1(IV) collagen gene. Subcloned fragments of this gene have been used to identify high frequency restriction fragment length polymorphisms. These are being used in attempt to link the α1(IV) collagen gene with putative type IV collagen disorders such as adult polycystic kidney disease and Alport's syndrome.

Retinoic acid, a well recognized human and animal teratogen, has been shown to reduce mRNA levels for type II collagen and increase mRNA levels for type III collagen in developing chondrocytes. These observations suggest that retinoic acid may act as a teratogen by altering the phenotype of the cells.

Biological Activities and Structure of Basement Membranes

Basement Membrane Matrigel - Laminin, a major component of basement membrane, has been found to have profound effects on adhesion and the growth of epithelial cells. We have also found that laminin promotes macrophage phagocytosis. We now find that extracts of the basement membrane can be reconstituted in vitro into a three-dimensional gel which is enriched in laminin and other basement membrane components including type IV collagen, entactin, nidogen, and heparan sulfate proteoglycan. This gel affects cell differentiation. When hair follicles primordia, composed of 6-8 cells, are isolated from newborn mice and embedded in the matrigel they grow and differentiate. By one week in culture, a hair shaft is visible. In contrast, on type I collagen gels or on plastic substrates these follicles dedifferentiate into a monolayer of cells. Another cell type with large responses to the basement membrane matrigel is the Sertoli cell which is fifteen-fold more columnar when grown on top of the gel. When grown inside the gel, Sertoli cells reorganize into cord-like structures which produce three-fold more androgen binding protein, support germ cell maturation, and survive upon subsequent implantation in animals. Various other cells including thyroid follicles, Schwann cells, heptocytes, mesangial capillary cells, and type II pneumocytes respond with maintenance of their phenotype and/or improved growth and differentiation. In addition, the matrigel in vivo has been found to promote regeneration of optic and sciatic nerves, and spinal cord. The axonal growth is faster and can occur over much greater distances than has been previously achieved with any other medium.

Tumor Cell Invasion Through the Basement Membrane - Malignant tumor cells degrade and pass through basement membrane whereas benign tumor cells do not. Previously we found that malignant tumor cells have a high affinity for basement membrane and in particular for laminin. We have prepared a reconstituted basement membrane from laminin and type IV collagen and used it in an _in vitro_ assay. The matrix is a barrier to the passage of benign tumor cells through which metastatic cells can pass. Cells crossing the barrier were found to be more metastatic than the parent population. In addition, the assay has been used to demonstrate that tumor cells home to specific organs due to protein chemoattractants produced by the tissue. For example, tumor cells, which normally metastasize to liver, were found to penetrate in greater numbers through the basement membrane barrier if extracts of liver were present on the opposite side than if extracts of lung, brain, or muscle were present.

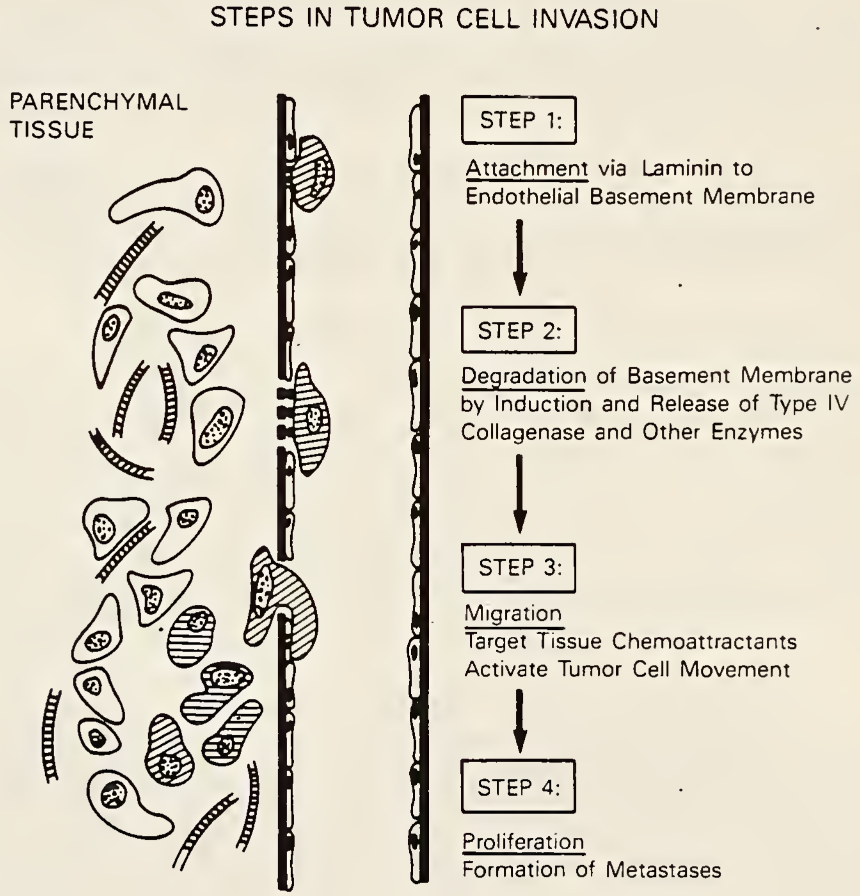
STEPS IN TUMOR CELL INVASION
PARENCHYMAL TISSUE
STEP 1:
Attachment via Laminin to Endothelial Basement Membrane
STEP 2:
Degradation of Basement Membrane by Induction and Release of Type IV Collagenase and Other Enzymes
STEP 3:
Migration
Target Tissue Chemoattractants Activate Tumor Cell Movement
STEP 4:
Proliferation
Formation of Metastases

Figure 2. Steps in tumor cell invasion.

Our data suggest that a specific sequence of events occurs when the tumor cells penetrate the basement membrane (Figure 2). The tumor cells first attach via laminin to the basement membrane. The binding of the cells to the basement membrane induces the cells to produce proteases and collagenases that degrade the basement membrane. The tumor cells then respond to local factors in the tissue which cause the cells to migrate and to proliferate in the tissue.

Structure of Basement Membrane Heparan Sulfate Proteoglycan

The basement membrane proteoglycan has a 400,000 MW core protein with heparan sulfate side chains. Considerable progress in determining the structure of this proteoglycan has been made by using a combination of electron microscopic Kleinschmidt spreading techniques and peptide mapping. Electron microscopic examination showed the proteoglycan to contain a 180 nm long core protein with 4-6 side chains 28 nm long located on one half of the core protein. Trypsin digestion of the proteoglycan produced a 200,000 MW peptide which was shown not to contain heparan sulfate. This peptide is likely from the region of the core protein found by electron microscopic examination to be devoid of side chains. Digestion of the proteoglycan with V-8 protease produced two major peptides of 41,000 and 42,000 MW, both of which also lack heparan sulfate. These observations suggest that the proteglycan is assymetric with over half of the core lacking side chains.

Molecular Interactions - Using the rotary shadow technique in electron microscopy, the structure and interaction of various basement membrane components have been assessed. For example, the interaction of laminin with Clq has been observed by both chemical assays and rotary shadowing. The tails of the Clq bind to the short arms of laminin. Such interactions may be important in the deposition of immune complexes in basement membranes in certain diseases.

Type IV collagen has two binding sites for laminin, one for heparan sulfate proteoglycan and one for fibronectin all of which are located at different sites. These and other data confirm that the molecular interactions are specific and may be involved in the assembly of the tissue in vivo. Using computer analyses, it is possible to account for all the interactions in a model of the basement membrane.

Mitoattractants - Previously, Dr. Grotendorst of LDBA identified the platelet-derived growth factor (PDGF) as a chemoattractant for mesenchymal cells. Since PDGF also was a potent mitogen for these cells, it appeared that PDGF exerted dual activity and was a member of a new class of biological mediators, the mitoattractants. PDGF is a wound hormone since it is released from aggregated platelets. Our in vivo studies have shown that the healing of experimental wounds is accellerated by the addition of PDGF but not by other growth factors. We also find that the rate of bone induction in vivo is increased. The possibility that PDGF will improve fracture healing is under study. Further studies will concentrate on the genes active in fracture healing.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00006-25 DB

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemotaxis in Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Schiffmann, Elliott	Research Chemist	NIDR DB

COOPERATING UNITS *(if any)*

NCI, NIH;

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	-

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying biochemical events characteristic of malignant tumor cells, which must be highly motile while invading tissue and metastasizing to distant sites. We have found that a number of metastatic cell lines produce and respond to autocrine motility factors. A partially purified material from the conditioned media of a human melanoma cell line was found to be a protein with an Mr of about ~55 KD. The material induces a strong chemotactic response in the producer cells and appears to exert its action by perturbing membrane phospholipid metabolism of the cell. We have extended these observations to 3T3 cells and their transformed metastatic counterparts. We find that the transformed cells produce and respond well to autocrine factors but poorly to platelet-derived growth factor (PDGF). The nontransformed cells, on the other hand, respond well to PDGF but to a lesser extent to the autocrine factors of the transformed cells. The nontransformed cells do not produce autocrine motility factors. These results suggest that an important characteristic of the metastatic phenotype of malignant cells is their ability to produce and respond to autocrine motility factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00009-24 DB

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry and Biosynthesis of Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin, George R.	Ch, Lab. Dev. Bio. & Anomalies	NIDR DB
Kleinman, Hynda K.	Research Chemist	NIDR DB
Howes, Robert	Guest Researcher	NIDR DB
Bowness, Michael	Guest Researcher	NIDR DB
Albini, Adriana	Visiting Associate	NIDR DB
Bolander, Mark	Staff Fellow	NIDR DB
Martinet, Nadine	Visiting Fellow	NIDR DB
Smith, Lyman	Guest Researcher	NIDR DB

COOPERATING UNITS *(if any)*

Max-Planck-Institute for Biochemie, Germany; McGill University, Canada; University of Texas, San Antonio, Texas

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.23	7.38	2.85

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the formation, function and the destruction of connective tissue components in normal and diseased states. Particular attention is directed toward various matrix molecules including collagens, glycoproteins, and proteoglycans. Current aspects of this project include (1) the role of the extracellular matrix in acquired and inherited disorders, (2) DNA changes in various pathological conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00024-19 DB

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental processes in genetically controlled malformations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Kenneth S.	Medical Director	NIDR DB
Campbell, Marlissa	Postodoctoral Fellow	NIDR DB
Harne, Leslie C.	Bio Lab Technician (Animal)	NIDR DB

COOPERATING UNITS *(if any)*
Howard University; University of Maryland; University of Washington, Seattle; NCI, NIH; NEI, NIH; NIAMDD, NIH; NIEHS, NIH; and USDA Poison Plant Laboratory

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.58	1.95	4.63

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are using mice with genetic defects in craniofacial development or differences in susceptibility to teratogens as models to study the mechanisms involved in craniofacial malformation. Mutations that cause cleft lip, cleft palate, malocclusion and exencephaly have been found to have chondrocytes that produce altered cartilage matrix. Several different genetic defects in matrix biochemistry result in similar malformation syndromes. Other mutant genes reduce or render defective essential cellular processes such as keratinization or other types of cell differentiation with resultant malformation. Other genes that modify the action of teratogenic drugs are used to explore the mechanisms of teratogenesis and to develop sensitive test systems for recognition of teratogens. These mouse genetic test systems are produced using uniform, highly inbred, genetically defined strains and timed mating resulting in control of stages of development for tests of target tissues at critical periods of susceptibility in vivo or in vitro. Organs or organ primordia such as limb buds or cells from embryos of appropriate genetic type and developmental stage are labeled in vivo or in vitro with isotopic precursors for specific structural molecules in order to determine the mechanisms of gene action in development and the mechanisms of teratogenesis.

These methods have proven successful in identification molecular defects in cartilage matrix and other specific molecular errors that result in malformation. We are continuing to use this approach to determine the molecular vocabulary of development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00025-19 DB

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of connective tissue gene expression during development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Yamada, Yoshihiko	Visiting Scientist	NIDR DB
Doege, Kurt	Postdoctoral Fellow	NIDR DB
Sakurai, Yoshiki	Visiting Fellow	NIDR DB
Kohno, Kimitoshi	Visiting Associate	NIDR DB
Sasaki, Makato	Visiting Fellow	NIDR DB
Iwamoto, Yukihide	Visiting Associate	NIDR DB
Rhodes, Craig	Biologist	NIDR DB
Horton, Walter	Postdoctoral Fellow	NIDR DB

COOPERATING UNITS *(if any)*

Max-Planck Institute for Biochemie; Genex Corporation; John Hopkins University;

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11.49	7.90	3.59

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. We have isolated a cDNA clone encoding the rat cartilage link protein. The 800 bp cDNA was sequenced and found to have two homologous stretches of 19 amino acids. This region also shows strong homology to the reported amino acid sequence of the bovine cartilage proteoglycan, suggesting a common role for these sequences in binding to link and proteoglycan. 2. We have isolated cDNA clones for the mouse laminin A, B_1, and B_2 chains. The nucleotide sequence of a 5.5 Kb cDNA insert for the B1 chain was determined. Computer analysis of predicted protein secondary structure suggests that the B1 chain has at least four distinct structural domains. 3. We have isolated a series of overlapping genomic clones which span about 60 Kb encoding most of the B1 chain of mouse laminin. The gene structure was examined by electron microscopic analysis. The gene shows an unusual clumping of small exons separated by large introns. 4. We have isolated a series of overlapping genomic clones for the human type II collagen. The nucleotide sequence of the promoter of this gene shows a strong homology to those of the rat type II collagen gene. 5. We determined the nucleotide sequence of eight exons coding for the helical part of the mouse α1 type IV collagen. The size of these exons suggest that the gene for α1 type IV collagen evolved separately from the interstitial collagen genes. 6. We have constructed recombinant plasmids in which type II collagen promoter determines the level of an E. coli enzyme, chloramphenicol acetyltransferase. Transfection of these plasmids to chick chondrocytes reveals that the sequence upstream from -380 is necessary for the efficient transcription of the type II collagen gene.

Kleinman, Hynda K.	Research Chemist	NIDR DB
Martin, George R.	Ch, Lab. Develop. Biol. & Anomal.	NIDR DB
Fernandez, Pilar	Visiting Fellow	NIDR DB
Killen, Paul	Staff Fellow	NIDR DB
Selmin, Ornella	Visiting Fellow	NIDR DB
Nunez, Anne Marie	Visiting Associate	NIDR DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00149-11 DB

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alterations in Proteoglycans During Abnormal Development and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hassell, John R.	Research Biologist	NIDR DB
Kleinman, Hynda K.	Research Chemist	NIDR DB
Horton, Walter	Postdoctoral Fellow	NIDR DB
Leyshon, Webster	Biologist	NIDR DB
Mosley, General	Biol. Lab Tech.	NIDR DB
Horigan, Elizabeth	Biologist	NIDR DB

COOPERATING UNITS *(if any)*

Upjohn, Kalamazoo, Michigan

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.45	3.20	4.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the structure of proteoglycans as well as to understand the role that they play in the function of tissues and during developmental events. We are characterizing functional domains of tissue specific proteoglycans by isolation and characterization of peptide fragments derived from proteolytically cleaved proteoglycan. Genetic clones of proteoglycans are being isolated by screening expressing vectors with antibodies directed against the core protein of the proteoglycan. Particular attention has been directed toward studying the heparan sulfate proteoglycan of basement membranes which we have found to contain a basement membrane specific 400,000 MW core protein. We have isolated a heparan sulfate free peptide of 200,000 MW from the proteoglycan. These observations, in combination with electron microscopic examination of the intact proteoglycans, indicate the heparan sulfate side chains are located asymetically, on only one region of the core protein.

We are also using chondrocytes to determine the mechanisms by which teratogens disrupt the synthesis of proteoglycans and other matrix components during development. Retinoic acid, a teratogen which produces limb and facial malformations in vivo is also known to disrupt chondrogenesis in vitro. We have found that retinoic acid inhibits the synthesis of type II procollagen, and cartilage proteoglycan core protein, while stimulating the synthesis of type III collagen and fibronectin. Furthermore, these alterations in protein synthesis are due to changes in mRNA levels for the proteins. These data suggest that retinoic acid acts to change the phenotype of the chondrocytes, possibly by affecting transcriptional activity.

Noonan, Douglas	Postdoctoral Fellow	NIDR DB
Yamada, Yoshihiko	Visiting Scientist	NIDR DB
Laurie, Gordon	Visiting Fellow	NIDR DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00230-09 DB

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of extracellular matrix proteins in tissue architecture and cell function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kleinman, Hynda K.	Research Chemist	NIDR DB
Chandrasekhar, S.	Guest Researcher	NIDR DB
Martin, George R.	Ch, Lab. Dev. Biol. & Anomalies	NIDR DB
Hassell, John	Research Biologist	NIDR DB
Laurie, Gordon	Visiting Fellow	NIDR DB
Cannon, Frances	Biologist	NIDR DB
Kitten, Gregory T.	Postdoctoral Fellow	NIDR DB

COOPERATING UNITS *(if any)* NIAID, NIH; NCI, NIH; NINCDS, NIH; University of Minnesota; Georgetown University; McGill University, Montreal, Canada; Rutgers University; Harvard University; Texas Tech, Lubbock, Texas; University of Missouri; Kalnins; Univ. of Toronto, Canada; Upjohn, Kalamazoo; Eli Lilly Co., Indianapolis, Indiana

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 8.85	PROFESSIONAL: 5.45	OTHER: 3.45

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the structure and function of extracellular matrix components. Molecular approaches have been used to probe the structure of certain molecules and well as to characterize active domains. In vitro studies have been used to understand the molecular interactions involved in matrix assembly. Our data define how the collagens, proteoglycans and glycoproteins interact with each other to form supramolecular complexes which ultimately assemble to form the mature matrix. Such interactions are important in determining the final structure and function of tissues and likely play an important role during tissue morphogenesis, in wound repair and in pathological states.

Various biological activities of epithelial cells are controlled by basement membrane components. Using three-dimensional cell culture, striking effects on cell growth and differentiation have been observed with basement membrane components. Hair follicles from embryonic mice are found to differentiate and produce a hair shaft while Sertoli cells become 15-fold more columnar and germ cell survival and differentiation are enhanced. The Sertoli cells reform tube-like structures when grown inside the three-dimensional matrix. Nerve cell sprouting and growth are significantly improved in vitro and in vivo when a basement membrane gel is placed in contact with the neurons. These and other studies with cultured cells and organs indicate that matrix components may regulate tissue formation by promoting cell growth and differentiation.

PHS 6040 (Rev. 1/84) GPO 914-918

Kolega, John	Postdoctoral Fellow	NIDR DB
Raimondi, Laura	Visting Fellow	NIDR DB
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00275-07 DB

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological testing of fluoride

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin, George R.	Ch. Lab. Dev. Biol. & Anomalies	NIDR DB
Brown, Kenneth S.	Medical Director	NIDR DB

COOPERATING UNITS *(if any)*

University of Minnesota; NCI, NIH

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the action of fluoride in various biological tests used to detect clastogenic or mutagenic substances. To date, fluoride has been examined in tests used to detect mutagens and found to be non mutagenic. No effects on chromosome structure were noted in animals given widely different levels of fluoride. DNA repair after X-ray was unchanged by fluoride. No genetic effects of fluoride were noted in a recessive lethal test of fluoride on Drosophila. The data indicate that fluoride has no mutagenic activity. Reports of fluoride's action on the metabolism and growth of cells are being monitored and where appropriate, related studies are undertaken.

ANNUAL REPORT OF THE LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY
NATIONAL INSTITUTE OF DENTAL RESEARCH
October 1, 1984 to September 30, 1985

During the past year, two principal investigators in the Laboratory of Microbiology and Immunology were honored for their sustained productivity and excellence in research. Dr. Sharon M. Wahl was the recipient of an NIH Director's Award for her research on the role of cellular immunity in chronic inflammatory diseases and for superior leadership as Chief of the Cellular Immunology Section. Dr. Bruce M. Chassy received the Special Recognition Award from the U.S. Public Health Service for his innovative work on developing recombinant DNA technology to investigate the physiology and pathogenic potential of oral bacteria. Several of our scientists participated as invited speakers and discussants in national and international symposia and workshops and contributed numerous chapters and reviews in books and journals as well as to a monograph on "Molecular Basis of Oral Microbial Adhesion" which was published in February, 1985 by the American Society for Microbiology.

There have been no major changes in the program areas of this laboratory since last year's report. Considerable effort has been spent on strengthening collaborative research within the Laboratory and with outside institutions in order to carry out experiments in the most economical and expeditious fashion. This has been particularly rewarding in our cooperative studies with other Institutes at NIH on the pathogenesis of chronic diseases such as rheumatoid arthritis and the Acquired Immune Deficiency Syndrome as well as in our programs on molecular biology and microbial pathogenesis. These collaborations have become an absolute necessity because of limited personnel and constraints on our budget for supplies and equipment. Noteworthy advances have been forthcoming in several areas and these are summarized below according to section designations.

The *Microbiology Section* focusses on microbial ecology, biochemistry and molecular biology. The program seeks to explain physiological and pathological traits of oral microorganisms in molecular terms. The underlying theme of this program is founded on the premise that new knowledge accruing from fundamental inquiries will establish a basis for the formulation of rational approaches to the control or eventual eradication of oral diseases having a microbial etiology.

The Section's ecology program continues its investigations on intergeneric coaggregation reactions that occur among diverse members of the oral microbial flora and which are now widely accepted to be of major importance in the development of dental plaque. These highly specific cell-cell recognition reactions are mediated by lectin and complex carbohydrate molecules located on the cell surfaces of the interacting bacteria. Past studies were devoted to conducting extensive surveys of oral bacteria that were designed to assess the scope and specificity of the coaggregation reactions and to test various models that were proposed to account for the diverse types of interactions observed. We are now concentrating on the isolation and chemical characterization of certain cell surface lectins that mediate some of the coaggregation reactions.

Last year we reported that certain strains of *Bacteroides loeschei* act as coaggregation bridges between non-coaggregating pairs of bacteria. For example, *Streptococcus sanguis* C104 and *Actinomyces israelii* PK14 had no ability to coaggregate with one another. However, each coaggregates with *B. loeschei* PK1295. *S. sanguis* and *A. israelii* interact with different *B. loeschei* lectin-like surface structures. The *S. sanguis* reaction was inhibited by lactose while the *A. israelii* reaction was not. Moreover, antiserum prepared against whole cells of *B. loeschei* prevented both coaggregations. When the antiserum was extensively adsorbed with whole cells of a *B. loeschei* mutant defective in its ability to coaggregate with *S. sanguis*, the ability to block the *B. loeschei* - *S. sanguis* coaggregation was unaffected, while the ability to block the *B. loeschei* - *A. israelii* coaggregation was lost. Mutants with a coaggregation-defective phenotype have now been isolated for all three organisms. These mutants and antisera prepared against them are being used to purify and chemically characterize the surface lectins involved in the coaggregation bridging reactions.

Significant progress has also been made in efforts to characterize a cell surface lectin on *Capnocytophaga gingivalis* that mediates its coaggregation with *A. israelii* PK16. The molecule of interest has been located on the *Capnocytophaga gingivalis* outer membrane. It is a basic protein (P.I. 8.4) with a subunit molecular weight of 155,000 daltons. Several lines of evidence indicate that the isolated polypeptide is the coaggregation mediating lectin. Outer membrane preparations from several independently isolated coaggregation defective mutants were all missing the 155,000 dalton polypeptide. In addition, Western blot analysis showed that coaggregation blocking antiserum prepared against wild type cells and extensively adsorbed with mutant cells reacted with the 155,000 dalton protein on two dimentional SDS-polyacrylamide gels.

The structure of a third lectin present on the type 2 fimbriae of *Actinomyces viscosus* and previously shown to be responsible for the coaggregation of this organism with *S. sanguis* is being investigated by recombinant DNA techniques and these studies will be described later under our molecular biology program.

The ecology program has recently expanded its inquiries to include investigations of metabolic interactions among various members of the oral microbial flora. One such study deals with a model food chain system involving mixed cultures of *Streptococcus mutans*, *Veillonella rodentium* and one of several strains of methanogenic bacteria that have been isolated from human subgingival plaque. In this tripartite mixed culture system, *S. mutans* converted glucose to lactate and *V. rodentium* fermented the lactate to substrates (H_2 and CO_2) that were used by the methanogen for growth and the production of methane. We anticipate that these studies will eventually help to determine why certain metabolic products from individual microorganisms accumulate in dental plaque while others do not.

Our biochemical studies have centered on two fundamental aspects of microbial physiology: 1) mechanisms of carbohydrate and pentitol transport, and 2) mechanisms of protein turnover.

Prior work demonstrated that the vectorial transport of most fermentable sugars and pentitols by the lactic acid bacteria is mediated by a complex complement of enzymes known collectively as the phosphoenolpyruvate phosphotransferase system (PEP-PTS). We have found that growth of *S. lactis* on glucose is inhibited by certain non-metabolizable analogues of glucose and that growth of

L. casei on glucose is inhibited by xylitol which cannot be metabolized by glucose adapted cells. In both of these cases, growth inhibition is due to the operation of a phosphorylation-dephosphorylation futile cycle involving the glucose analogues and xylitol respectively. Phosphorylation and transport of the growth inhibitors is mediated by the PEP-PTS and their dephosphorylation is catalyzed by a cytosol phosphohydrolase. The phosphohydrolases are key enzymes in the operation of both futile cycles. They have been extensively purified from both organisms and shown to possess several unusual properties. For example, their activities appear to be regulated by a cell membrane component or perhaps by their actual physical association with the cytoplasmic membrane. Furthermore, when antiserum prepared against the purified enzyme from *L. casei* was used as a probe, at least 4 major bands of cross-reacting material were detected by immunoblotting of cell extracts following electrophoresis. The molecular weights of these components from *L. casei* were 82, 72, 32 and 17 K daltons. In *S. lactis*, three distinct proteins were isolated, all of which specifically hydrolysed hexose-6-phosphate. Interestingly, two of these proteins have a similar M.W. (36-37 K daltons) while the third has an apparent M.W. of 67 K daltons. The relationship between these different forms of the phosphatases is not yet clear. It is conceivable that: 1) they represent post-translational modifications of the same gene product, 2) that they have arisen by gene duplication and divergence, or 3) that they represent interconvertible forms of the same enzyme. These possibilities are currently under investigation.

We have also begun a study of carbohydrate transport in the oral anaerobe *Peptostreptococcus anaerobius*. This organism has been found to have an obligatory requirement for proline for the transport of glucose via the PEP-PTS. The specific requirement for an amino acid for the PEP-PTS mediated transport of glucose has never before been reported. Our current results indicate that proline serves as an acceptor for reducing equivalents generated during the conversion of intracellular alanine to acetyl-P and ATP. In this reaction, proline is reduced to delta-aminovalerate by a membrane bound proline reductase. The high energy phosphate compounds formed from alanine then serve either directly or indirectly as phosphate donors for the initial phosphorylation and translocation of glucose.

We have also continued our long term study on mechanisms of protein turnover in *S. salivarius* by investigating the biochemical reactions involved in the irreversible, copper ion-mediated inactivation of a membrane-associated fructosyltransferase (FT). The recent development of an *in vitro* FT inactivation assay has allowed us to unequivocally identify one of two NADH oxidase complexes present in this organism as the FT inactivating enzyme system. FT inactivation by NADH oxidase had an absolute requirement for copper or iron. In addition, the inactivation was completely prevented by H_2O_2 scavengers (catalase, pyruvate), and partially by superoxide dismutase and the hydroxyl radical scavenger mannitol. We have also demonstrated that the superoxide anion and H_2O_2 are both products of the NADH oxidase. It is not clear at this point whether the two dioxygen reduction products are formed by a single enzyme, or whether they arise from more than one enzyme present in the NADH oxidase preparation. A tentative mechanistic model that is fully consistent with the present data is as follows: FT initially binds Cu^{++} at or near the catalytic site. Superoxide ions produced by NADH oxidase reduce the FT-Cu^{++} complex to FT-Cu^{+}. The enzyme-bound Cu^{+} is then reoxidized by H_2O_2 (also produced by the NADH oxidase preparation) by a 'Fenton' type reaction to form hydroxyl radicals. We suggest that the highly reactive hydroxyl radical oxidizes a key amino acid residue (histidine?) at or near the catalytic site resulting in the irreversible inactivation of FT. We believe that this oxidative inactivation of FT represents a selective but not specific marking reaction that identifies the catalytically inactive protein as a substrate for one or more proteases.

Our molecular biology program has made significant advances in three areas: 1) the use of recombinant DNA techniques to identify and chemically characterize surface structures on oral bacteria that mediate specific coaggregation or adherence reactions, 2) the development of genetic exchange systems for oral lactobacilli, and 3) the study of lactose metabolic plasmids.

Last year, we reported the results of a collaborative study with the Humoral Immunology Section which led to the successful cloning of an *A. viscosus* gene in *E. coli* that expressed a 59 kDA protein subunit of the *A. viscosus* type 2 fimbriae. This structure carries the lectin responsible for mediating a specific and lactose reversible coaggregation reaction with *S. sanguis*. Our ultimate objective is to sequence this gene (*fimA*) in order to determine the primary structure of its 59 kDA protein product (P59). The cloned *fimA* gene, however, resided on a 50 kb plasmid (pAV1402), and before we could proceed with further characterization and sequencing it needed to be mapped and subcloned. Shotgun subcloning of pAV1402 has revealed that *BamHl* generated a 4.4 kb fragment (E) and a 1.8 kb fragment (F) which expressed proteins of 35 and 22 kDa respectively. The protein product of both fragments reacted with antibody against type 2 fimbriae. Other studies showed that the E and F fragments were contained within the 9.7 kb *HindIII* B fragment of pAV1402 and when the latter fragment was subcloned into pUC13 it expressed p59 strongly. A detailed restriction map of pAV1402 has been constructed which showed that the 9.7 kb *HindIII* B fragment was the smallest one that contained the entire *fimA* gene. This fragment was further mapped with various restriction enzymes to determine the relationship between the *BamHI* generated fragments (E and F) and to find suitable restriction sites for further subcloning. Results from these studies showed that the E fragment encodes the carboxy terminal and the F fragment the amino terminal of p59. To facilitate sequencing, *KpnI* fragments of 1.1, 1.8, and 1.0 kb were subcloned into pUC18. These fragments encode, respectively, the amino terminal, the carboxy terminal, and the remainder of p59. This work now sets the stage for sequencing *fimA*.

We reported previously the successful development of a liposome-mediated phage transfection system for introducing DNA into protoplasts of *L. casei*. The protoplasts were subsequently regenerated to whole cells and transfectants identified by scoring for phage plaques in agar overlays containing an indicator strain. This represented a major step in our long range goal to develop genetic exchange systems for this group of organisms. The liposome-DNA-protoplast system has now been used to transform cells with small *L. casei* cryptic plasmids previously constructed as cloning vectors. The system has also been used to introduce lactose plasmids isolated from *L. casei* into isogenic lactose non-fermenting mutants of the same organism. Interestingly, plasmids could not be isolated from these transformants and the genes encoding for lactose utilization appear to have been integrated into the chromosome. This work represents the first successful demonstration of transformation in the lactobacilli and it opens the way to study diverse phenotypic traits in this group of organisms at the molecular level.

We continue to use molecular biological approaches for studying certain metabolic genes and this work complements our biochemical studies on carbohydrate transport. For example, we have found that a large plasmid carried by *L. casei* encodes for Enzyme II^{lac} and Enzyme III^{lac} of the lactose: PEP-PTS as well as the lactose-P hydrolyzing enzyme phospho-β-galactosidase (P-β-gal). The gene encoding the latter enzyme has been cloned into *E. coli* and work on its sequence is nearly completed. The sequence of the *E. coli* gene is known and when the *L. casei* P-β-gal sequence is finished it will, for the first time, allow a comparison of the promotor regulatory sequences and structural homology of the enzyme produced by these two organisms. This should provide interesting information concerning promotor function and structural-catalytic relationships.

The research objectives of the *Humoral Immunity Section* are aimed at defining the molecular interactions which initiate bacterial attachment, colonization and the subsequent induction of inflammation of oral tissues. Since the ultimate destruction of oral tissues may be directly attributed to these processes, investigations have focused on identification and characterization of the bacterial surface structures which mediate adherence to tooth, epithelial and other bacterial surfaces as well as the purification of complementary receptors for microorganisms on other bacteria and mucosal surfaces. The response of the host to oral infection is multifaceted. Thus, anti-bacterial antibodies, complement and phagocytic cells all contribute to the control of the microbial flora but, in addition, are instrumental in the establishment and progression of inflammation. Therefore, current research efforts also encompass exploration of the production of anti-bacterial secretory antibodies, the potential effects of bacterial proteases on these antibodies and the interaction of oral bacteria with the complement system and phagocytic cells.

Two fimbrial adhesins have previously been identified on *Actinomyces viscosus*. One of these (type 1) mediates bacterial attachment to saliva treated hydroxyapatite (SHA) and presumably the tooth whereas the other (type 2) is associated with a lectin activity that recognizes specific carbohydrate containing receptors on other bacteria and mammalian cells. The initial finding that the type 1 fimbriae are involved in attachment to SHA has been considerably extended by the recent demonstration that antibodies monospecific for type 1 fimbriae react with all of 23 strains of *A. viscosus* and *Actinomyces naeslundii* that adsorb strongly to SHA and, in the majority of cases, these antibodies inhibit attachment. In contrast, the anti-type 1 fimbrial antibodies do not react with bacterial strains which adsorb poorly to SHA. Thus, the type 1 fimbriae are the principal adhesins mediating this interaction.

As indicated earlier, in collaboration with the Microbiology Section the application of recombinant DNA technology to the examination of the subunit structure of these fimbriae has resulted in the cloning of the gene encoding the type 2 fimbrial subunit. During the past year these techniques have also been used to clone the gene for the type 1 fimbrial subunit. The cloned gene product, which has been detected by both monospecific and monoclonal antibodies specific for type 1 fimbriae, has a molecular weight of 62,000. Subcloning has resulted in the identification of a 3 Kb fragment that encodes the entire fimbrial subunit.

The accumulation of dental plaque involves the coaggregation of bacteria of different species. One such interaction occurs between *S. sanguis* 34 and *A. viscosus*. A polysaccharide on the streptococci serves as a receptor for the type

2 fimbriae of the actinomyces. This polysaccharide has been purified and its structure determined. It consists of hexasaccharide chains linked by phosphodiester bonds. The purified hexasaccharide is composed of GalNAc, Gal, Glc and L-Rha with GalNAcα3Rha at the terminal nonreducing end.

The colonization of oral mucosal surfaces by actinomyces is also attributed to the interaction of the lectin associated with the type 2 fimbriae with a carbohydrate containing receptor on epithelial cell surfaces. Bacterial adherence to epithelial cell lines is inhibited by β-linked galactosides and is significantly enhanced by the exogenous addition of sialidase. Interestingly, the actinomyces secrete sialidase which exposes the receptors for the bacteria. This epithelial cell receptor has been partially characterized by the utilization of plant lectins of known specificities. The peanut agglutinin (PNA) which is specific for Galβ3GalNAc and the lectin from *Bauhinia purpurea* (BPA) which reacts with Gal, GalNAc inhibit bacterial attachment whereas numerous other plant and invertebrate lectins bind to the epithelial cells but do not inhibit bacterial adherence. Since the reactivities of these two plant lectins are similar to that of the actinomyces lectin, they have been used as probes for the epithelial cell receptor. PNA and BPA detect a band of 160 Kd on sialidase treated Western blots of epithelial cell extracts separated by SDS-PAGE. This 160 Kd component is a cell surface sialoprotein which can be surface labeled and is metabolically labeled with [^{14}C]GlcNAc. The epithelial cell receptor has been partially purified by ion exchange and lectin affinity chromatography. Interestingly, the evidence to date indicates that this receptor is similar but not identical to the receptor on *S. sanguis*. Whereas BPA detects receptors on both epithelial cells and streptococci, PNA recognizes receptors only on epithelial cells. Sialidase treated erythrocytes have been widely used as a model system for examining attachment of the actinomyces. Characterization of the erythrocyte receptor indicates that it is a glycolipid. Thus, both glycoproteins and glycolipids can serve as mammalian cell receptors for the actinomyces.

Investigation of host responses to the oral actinomyces has focused on the interaction of these bacteria with the complement system. Complement fixation by monoclonal antibodies to each of the fimbrial types has been used to localize epitopes on these fimbriae. These studies have provided evidence that the subunit structure of the type 1 fimbriae and the type 2 fimbriae differ. Thus, each of three monoclonal antibodies specific for the type 1 fimbriae in the absence of a second antibody activates the complement sequence. In contrast, only one of four monoclonal antibodies specific for the type 2 fimbriae (all of which consume complement when assayed in combination with a second antibody) activates this mediator system. Since complement activation by IgG antibodies is strictly dependent on the bridging of two antibody molecules by Clq within a given distance (approximately 20nm), the type 2 fimbriae are apparently more extended than the type 1 fimbriae, a finding which has recently been suggested by electron microscopic examination of the fimbriae. The biological activity of one monoclonal antibody can clearly be influenced by the addition of a second antibody. Combinations of monoclonal antibodies have revealed inhibition, enhancement, addition and synergy of complement activation.

Recent studies reveal that multiple mechanisms exist for the destruction of actinomyces by polymorphonuclear leukocytes (PMNs). The lectin associated with the type 2 fimbriae initiates rapid and effective killing of these bacteria by attaching to carbohydrate receptors exposed by sialidase treatment of PMNs. Thus, the parent strains of *A. viscosus* and *A. naeslundii* as well as an *A.*

viscosus mutant which possesses the type 2 but not the type 1 fimbriae are killed. In contrast, mutants which lack only the type 2 or both fimbriae remain viable after incubation with the sialidase treated PMNs. Polyvalent antibodies against A. viscosus or its mutant completely lacking fimbriae and antibodies monospecific for each of the two types of fimbriae were found to be extremely effective opsonins for phagocytosis of the actinomyces. These antibodies trigger phagocytosis through their interactions with the Fc receptors on PMNs. Marked enhancement of PMN mediated killing of bacteria sensitized with the polyvalent antisera by the addition of complement has been observed. This effect is attributable to the facilitation of binding of the bacteria to the PMNs by interaction of C3b (the large fragment of the third component of complement generated by activation of the complement sequence) or C3bi (C3b cleaved by the C3 inactivator and Factor H) with their complementary receptor(s) on PMNs. To date no complement enhancement of phagocytosis has been observed following complement activation by actinomyces sensitized with the monospecific anti-fimbrial antibodies. Further studies utilizing other antisera are currently in progress to localize the effective site(s) of C3b/C3bi deposition for enhancement of phagocytosis.

Studies are also continuing to examine another aspect of the host's response to oral microorganisms; namely the ability of secretory IgA to inhibit attachment of oral bacteria to other bacteria and epithelial cells and the effects of cleavage of the IgA by IgA proteases on these activities. Although the cleavage of myeloma IgAs by specific IgA proteases from several microorganisms has been well documented, virtually nothing is known concerning the biological activities of the resulting fragments. Pregnant primates (Macaca fascicularis) have been immunized with the type 1 fimbriae of A. viscosus and the type 2 fimbriae of A. viscosus and A. naeslundii and colostrum and milk have been obtained as the source of antigen specific IgA. Since previous investigations indicate a strict species homology between the sources of the bacterial isolates producing the proteases and the IgA and our studies confirm that monkey IgA is not susceptible to cleavage by the IgA proteases produced by human isolates, monkey isolates are being screened to identify bacterial species which secrete this potential virulence factor.

The Cellular Immunology Section continues its investigations into the mechanisms whereby mononuclear cells are recruited and activated to participate in inflammatory events. Cell mediated immune reactions are dependent upon lymphocyte-macrophage cooperation and the generation of soluble factors which exert profound influences on patterns of cell migration, inflammatory cell metabolism, cell surface properties and the activation of cells to participate in the biological activities involved in host defense. Although these lymphocyte and monocyte derived factors (lymphokines and monokines) have been defined mainly on the basis of in vitro assays, speculations concerning their in vivo importance have been and are being documented. Studies in the Cellular Immunology Section have provided evidence for the role of lymphokines in the development and maintenance of chronic lesions through detection of lymphokines in inflammatory tissues and through suppression of in vivo reactions by anti-inflammatory agents. Furthermore, these findings have been extended and related to clinically relevant situations.

Directed migration of phagocytic cells from the peripheral blood is a key event in the host response to infection, injury or antigen deposition. Since monocyte migration into an inflammatory site is critical to the outcome of the lesion, several investigators in the Section are exploring mechanisms of monocyte chemotaxis. Binding of chemoattractants to a receptor on the monocyte surface is presumably the first step in the molecular mechanisms of chemotactic activation. Members of the Cellular Immunology Section have, in collaboration with investigators at NMRI and UCSD, California, developed a red fluorescent probe for the synthetic bacterial analog, FMLP, and a green fluorescent probe for the chemotactic complement fragment, C5a, to enable simultaneous analysis of the expression of these two receptors on an individual cell which has not previously been possible. In characterizing peripheral blood monocytes, it was found that the majority of OKM1, MAC1, Fcγ receptor positive monocytes co-expressed receptors for C5a and FMLP. However, an unanticipated subpopulation of peripheral blood mononuclear cells was identified which expressed C5a but not FMLP receptors. After extensive studies, it became clear that these cells belonged to the natural killer (NK) cell population. These exciting observations implicate a role for complement-mediated recruitment of NK cells in host resistance to tumors and virus infected cells.

With the availability of these two different color fluorescent probes for chemotactic ligands, it also becomes possible to determine the mechanisms of normal monocyte chemotactic responsiveness and to probe the abnormal monocyte responses which have been reported in immunodeficiency diseases including the acquired immune deficiency syndrome, certain neoplastic diseases and in chronic inflammatory lesions. In this regard, monocytes from synovial fluid of rheumatoid arthritis patients migrate to FMLP, but not to C5a. Upon further analysis we found that these inflammatory monocytes while expressing a normal complement of FMLP receptors are markedly deficient in C5a receptors. During the course of studies to determine whether the synovial monocytes represented a subpopulation of C5a receptor deficient cells or were desensitized *in vivo*, evidence accumulated demonstrating that monocytes upon activation can differentially regulate their receptors for chemotactic stimuli. These observations may provide insight into mechanisms of recruitment and subsequent maintenance of a monocyte infiltrate at an inflammatory site.

In addition to the well characterized inflammatory mediators which stimulate monocyte chemotaxis, new studies demonstrate that neuropeptides, small signal peptides traditionally believed to function as transmitters of nerve impulses in the brain to mediate mood and emotion, can induce monocyte migration. Opiate peptides, enkephalin and β-endorphin, at concentrations as low as 10^{-14} M stimulate monocyte chemotaxis and the specificity of this activity can be demonstrated through the use of appropriate agonists and antagonists. In addition to the opiate peptides, the hypotensive, bradykinin-like peptide, substance P, and the hypothermic peptide, bombesin, exhibit this unique ability to trigger directed monocyte migration. Pharmacologic studies indicate that these chemotactic effects are indeed mediated by specific neuropeptide receptors on the monocytes. While the physiological correlate of *in vitro* neuropeptide induced monocyte chemotaxis is still obscure, the local release of neuropeptides may have important effects on cell distribution and function. The recent demonstration that substance P can exacerbate experimental arthritis and conversely, that local depletion of substance P can ameliorate joint inflammation provides impetus for continued examination of the role of these neuropeptides in inflammation.

Research in the Cellular Immunology Section also encompasses events subsequent to mononuclear cell recruitment including mechanisms of activation and the generation of mediators of inflammation. In this regard, a significant advance has been made by members of the Section who have established for the first time, specific receptors for γ interferon (γIFN) on human monocytes. Using a radioiodonated recombinant γIFN, these studies demonstrated that γIFN binds in a highly specific and saturable manner with high affinity to human monocytes. The number of binding sites was determined to be 3,000-5,000 per cell and no cross reactivity with α or βIFN was evident. Subsequent to γIFN binding to this specific receptor, monocytes express microbicidal and tumoricidal activity, and demonstrate enhanced numbers of Fc and HLA-DR antigens. Activation of macrophages by lymphokines such as γIFN contributes to a cascade of additional biologically active mediators. Activated human monocytes have been shown to produce immunoregulatory molecules including IL-1 and prostaglandin as well as enzymes such as collagenase. Since monocytes accumulate in large numbers at chronic inflammatory sites, these cells may be instrumental in the connective tissue destruction associated with these lesions as is evident in arthritis and periodontal disease. Furthermore, potentially important therapeutic information has been obtained by the ability to inhibit synthesis of this destructive enzyme with pharmacologic levels of certain anti-inflammatory drugs. The corticosteroid, dexamethasone, and the nonsteroidal anti-inflammatory agent, indomethacin, both inhibit production of collagenase. Furthermore, retinoic acid, currently being utilized to treat arthritis patients, blocks the generation of this enzyme. These observations indicate that amelioration of chronic inflammation-related connective tissue destruction by anti-inflammatory drugs may be, in part, the consequence of their ability to inhibit collagenase production.

In addition to the direct attack by monocytes on the connective tissue matrix, mononuclear cells can also indirectly mediate connective tissue changes. Since rheumatoid arthritis (RA) involves both proliferation of connective tissue components and destruction of the connective tissue matrix, this disease model is ideally suited to our analyses of the mechanisms whereby inflammatory mononuclear cells regulate both of these pathophysiologic changes in connective tissue. Through our studies which focus on the contributions of lymphokines and monokines to the immunopathology of the synovial lesion, we have recently provided significant new evidence that the accumulation of mononuclear cells in the synovial fluid and tissues to a large extent is T lymphocyte dependent. Synovial tissue from one subpopulation of arthritis patients produces copious amounts of a lymphocyte-derived chemotactic factor (LDCF) and is characterized by a heavy mononuclear cell infiltrate whereas synovia from another subpopulation which has minimal inflammatory cell infiltrate produces little or no LDCF. Of greater interest and importance is the striking evidence that therapeutic interruption of LDCF generation inhibits mononuclear cell recruitment and is associated with marked clinical improvement in these patients.

Because of the limitations associated with studying human disease, the studies of inflammatory cell regulation of connective tissue metabolism have been extended using an experimental model. The intraperitoneal injection of streptococcal cell wall (SCW) fragments into susceptible rats induces acute and chronic polyarthritis and hepatic granuloma and provides an ideal model for studying inflammation associated alterations in connective tissue. The role of T cells and their products was investigated in two T cell defective systems: congenitally athymic (nude) rats and cyclosporin A (CsA) treated rats. In both athymic and CsA treated animals, SCW induced acute lesions but these animals failed to develop chronic synovitis or granuloma. In contrast to earlier reports, these studies provide compelling evidence for the role of T lymphocytes in these lesions and in the marked alterations in connective tissue. Furthermore, these experimental observations have provided useful information concerning therapeutic intervention currently being applied to human rheumatoid arthritis patients.

While inflammatory cell regulation of fibroblast growth and function may be beneficial in normal wound healing and repair, in chronic inflammation the prolonged release of these mediators may lead to pathologic changes in connective tissue. Consequently, it is important to define these pathways in anticipation of their regulation. *In vitro* and *in vivo* work has demonstrated T cell dependent regulation of fibroblast growth and function. In order to prove that a putative T cell product has a physiologic role, it is necessary to purify the molecule and to produce specific antibodies to it. Then the consequences of neutralization of the endogenous molecule *in vivo* and thereby its physiological role can be established. Fibroblast activating factor (FAF) which stimulates fibroblast proliferation has been purified at least 5,000 fold and in addition, FAF poly A^+ mRNA from T lymphocytes has been translated in the Xenopus oocyte system. These recent advances will enable cloning of the FAF gene using recombinant DNA technology. With the recent recruitment of a molecular biologist into the Cellular Immunology Section, these and other important studies will be emphasized and expanded. Further characterization of the mechanism of FAF action on fibroblast growth revealed that FAF is unique in possessing both competence and progression activity. Additional analysis of the FAF-target cell interaction involves the generation of monoclonal antibodies to FAF specific receptors on the fibroblasts. Identification of fibroblasts which specifically react to T cell derived FAF may enable pharmacologic manipulation of the fibroplastic response associated with inflammation.

Definition of normal mechanisms of mononuclear cell recruitment, activation and effector function provides a baseline for comparison in disease. Whereas rheumatoid arthritis provides a clinical setting in which to explore the effects of prolonged and excessive mononuclear cell activation and mediator production, another clinically relevant study, the acquired immunodeficiency syndrome (AIDS), has afforded us the opportunity to determine the consequences of inadequate and ineffective mononuclear cell function. Investigators in the Cellular Immunology Section have documented that monocytes from AIDS patients are markedly defective in their ability to chemotax, kill microorganisms, generate inflammatory mediators including IL-1 and PGE_2 and present antigen. Since the monocyte is a key cell in the host's defense against viral infections, bacteria, parasites and tumor cells, the inability of these cells to participate normally in the elimination of these life-threatening agents likely contributes to the rampant infections and unrestricted growth of Kaposi's sarcoma which characteristically lead to morbidity and mortality in AIDS patients.

A major breakthrough in these studies is the recent identification of the monocyte as a target for the HTLV-III virus which is the presumed etiologic agent of AIDS. The ability of the monocytes to become infected and to harbor the virus likely provides a mechanism for the suppressed monocyte activities. In parallel studies, cytomegalovirus (CMV) which is also commonly apparent in AIDS patients has been found to infect monocytes and to suppress their functional repertoire likely further contributing to the immunosuppression in this disease. Unlike the $OKT4^+$ lymphocytes which are killed and therefore eliminated by the HTLV-III virus, the monocytes are not killed, but only functionally impaired. Because of this dichotomy, the monocytes may hold the key to immunoenhancing therapy.

Adding credence to these observations are exciting new studies utilizing cells from homozygous twins, one with AIDS and one without AIDS. By replacing the AIDS brother's defective monocytes with monocytes from the healthy brother, it was possible to restore depressed lymphocyte responsiveness. Furthermore, exogenous IL-1 provided an immunoenhancing effect on lymphocyte proliferation. These findings confirm that defective monocytes contribute to the immune defect in AIDS and illustrate further approaches to immunomodulation.

The Clinical Immunology Section studies the release of inflammatory mediators from basophils and mast cells. Current experiments attempt to define the mechanisms involved in cell secretion and the different mediators released from the cells. A cloned subline of the rat basophilic leukemia cells (RBL-2H3) has been developed for these *in vitro* studies. The cells divide rapidly, grow attached to plastic and have surface IgG and IgE receptors similar to normal basophils. They contain histamine and serotonin, and although obtained from a tumor and are heteroploid in chromosomal number, these cells have many similarities to normal mast cells or basophils. The cells can be activated by crosslinking of the immunoglobulin receptors to release mediators like histamine, serotonin, prostaglandins and other arachidonic acid products. This release is an energy-requiring step blocked by metabolic inhibitors. Similar indirect information on release mechanisms has been obtained by the use of inhibitors which are thought to interfere with other cellular functions. However, the availability of large numbers of cultured rat basophilic leukemia cells allows direct biochemical study of the events which occur in cells following activation. Variants of rat basophilic leukemia cells have also been obtained which are incapable of histamine release. Biochemical characterization of the defect in these cells is useful for determining the sequence of events involved in cell activation and secretion. These events include receptor crosslinking, proteolytic-enzyme activation, phospholipid methylation, changes in membrane lipids, Ca^{2+} influx-efflux, phospholipase activation, release of arachidonic acid, protein phosphorylation and finally, secretion of histamine.

The phosphorylation of cellular proteins modulates many functions. Experiments evaluated the phosphorylation of cellular proteins during the secretory process in the rat basophilic leukemia cells (RBL-2H3). Initial studies analyzed the cell lysates by one dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Following stimulation with either the Ca^{2+} ionophore or an IgE-stimulus, there was increased phosphorylation of a number of proteins; the most prominent and consistent changes were in a band migrating at ~92kDa. The enhanced level of phosphorylation was dependent on the dose of antigen or the Ca^{2+} ionophore, and correlated with the extent of histamine release. The phosphorylation of this band was present less than 1 min after stimulation, reached maximal levels at 1 to 3 min, and decreased to background by 30 min. This phosphorylation required the presence of Ca^{2+} in the medium. Therefore, the maximal phosphorylation changes preceded the release of histamine from the cell. The phosphorylation of this 92kDa component was studied in 20 variant cloned cell lines with various defects in histamine release. Phosphorylation occurred in the absence of histamine or arachidonic acid release, indicating that it is not a consequence of the release process. It was also evident in the absence of $^{45}Ca^{2+}$ uptake by the cells. These findings demonstrate early activation of a Ca^{2+}-dependent kinase during histamine release from RBL-2H3 cells resulting in the phosphorylation of the ~92kDa protein. The analysis of the phosphorylation products by 2-dimensional analysis (O'Farrell) allowed the recognition of about 30 other proteins which are phosphorylated during histamine release in the rat basophilic leukemia cells. Similar proteins were labeled following both IgE- and ionophore-mediated reactions. A number of these phosphorylations occurred in the absence of Ca^{2+} suggesting that they are biochemical events preceding the opening of the calcium channel. Analysis of these changes in mutants will help understand the order in which these phosphorylations occur within the cell and their possible significance for the secretory events.

One of the major biochemical events during basophil/mast cell activation is the rise of intracellular calcium [Ca^{2+}]. Measurements of this change can be made with the use of either the fluorescent dye quin-2, or radiolabeled $^{45}Ca^{2+}$. The use of quin-2 dye has become useful for cytoplasmic Ca^{2+} measurements. However, such determinations have two inherent problems; the intracellular concentration of quin-2 is high during these experiments. First, these high levels chelate part of the calcium entering the cell. Second, we have found that the formaldehyde generated by the esterase on the entry of quin-2 into cells decreases the secretory function of mast cells. We have thus concentrated on the use of $^{45}Ca^{2+}$ measurements in the rat basophilic leukemia cells. The incubation of the cells with this label requires at least 20-30 min for the cell associated $^{45}Ca^{2+}$ to reach a steady level. Therefore, after 60 min of incubation with $^{45}Ca^{2+}$, the effect of either IgE- or calcium ionophore A23187 stimulation was studied. Two independent events were observed; when the Ca^{2+} added to the buffer was 1.0 mM, cell activation resulted in a rapid increase in the intracellular $^{45}Ca^{2+}$ due to a net influx of calcium. When the Ca^{2+} added to the buffer was 0.1 mM, no influx of $^{45}Ca^{2+}$ was observed following cell activation, but there was a decrease in the cell- associated $^{45}Ca^{2+}$. This decrease in the cell-associated $^{45}Ca^{2+}$ was also observed in mutants of the RBL-2H3 in which there was no rise in intracellular Ca^{2+} concentration. A number of other experiments separated the decrease in the cell-associated $^{45}Ca^{2+}$ from the elevation seen following stimulation. La^{3+} blocked the rise in $^{45}Ca^{2+}$ but had no effect on the fall. Similarly, the decrease in the $^{45}Ca^{2+}$ was seen in a surose- buffered medium in which no increases were observed. Therefore, cell stimulation results in calcium efflux independent of any increased intracellular calcium or histamine release. Calcium efflux is a newly described biochemical event occurring early in cell activation.

The bridging of IgE receptors on rat basophilic leukemia cells (RBL-2H3) results in a number of biochemical events which accompany histamine secretion. Prominent among these is the release of arachidonic acid from cellular phospholipids which could be due to the activation of phospholipase enzymes. Experiments during this year were aimed to better define the phospholipid changes, the source of the arachidonic acid and the phospholipase enzymes activated during the secretory process. We studied the source of this arachidonic acid released following IgE- or calcium ionophore A23187-stimulation. A 48 hr culture of the cells with [^{14}C]-arachidonic acid resulted in labelling of the phospholipids to constant specific activity. Following IgE stimulation 8.8% of the cellular [^{14}C]-arachidonate was released; this was predominantly from phosphatidylinositol/phosphatidylserine with some from phosphatidylethanolamine and a minimal contribution from phosphatidylcholine. In contrast, following ionophore stimulation the cells released 16.4% of cellular [^{14}C]-arachidonate, with most of this coming from phosphatidylethanolamine, with much less from phosphatidylserine/phosphatidylinositol and phosphatidylcholine. Therefore, the source of the released arachidonic acid depends on the stimulus used to activate the cells. In contrast, the results are different when the cells are cultured for only 2 hr with [^{14}C]-arachidonic acid. The label in phospholipids is mainly found in phosphatidylcholine, with less in the other phospholipids. Stimulation of the cell with both IgE- or ionophore results in the release of the [^{14}C]-arachidonate predominantly from phosphatidylcholine. This suggests the presence of different pools of phospholipids which are labeled at different rates and/or have variable proximity (or accessibility) to the phospholipase(s) enzymes(s) activated during cell secretion.

In other experiments we studied the intracellular activation of phospholipase A_2 (PLA_2) during histamine release. RBL-2H3 cells were stimulated through the IgE-receptor, and cell homogenates prepared and tested for PLA_2 activity. The amount of this activity was dependent on the concentration of secretagogue used to activate the cells. Under optimal conditions there was a 2-fold increase in the activity found in stimulated cells. Activity was present in homogenates early following cell activation, and preceded histamine release. The activation of the enzyme in the cell occurred in the absence of extracellular Ca^{2+} in the medium, although the enzymatic activity required Ca^{2+}. The PLA_2 activity in the homogenates and the extent of cell stimulation for histamine release were maximal at the same concentration of antigen and both were blocked by the addition of a monovalent hapten. The enzyme in the homogenates was capable of cleaving arachidonic acid from different phospholipids. The production of lysophospholipids could play a critical role in histamine release from cells. These results demonstrate the activation of PLA_2 enzyme in cellular homogenates during the secretory process.

Steriods are important anti-inflammatory agents. There has been much interest in the phosphatidylinositol pathway as the intracellular signaling mechanism. IgE-receptor, but not A23187 ionophore-stimulation results in phosphatidylinositol breakdown in RBL-2H3 cells (Beaven, M. et al. J. Biol. Chem. 259, 7137, 1984). Experiments evaluated the effect of dexamethasone on these biochemical events following IgE-receptor-or ionophore-stimulation. Cells cultured for 8-24 hr with dexamethasone resulted in inhibition of IgE- and ionophore- mediated secretion. The removal of the dexamethasone after 24 hrs of culture resulted in a slow recovery of the histamine releasing activity. In dexamethasone treated cells, ^{45}Ca-influx was blocked following IgE receptor stimulation as was the release of [^{14}C]-arachidonic acid. Dexamethasone inhibited the IgE receptor- mediated phosphatidylinositol breakdown indicating inhibition of phospholipase C. However, as most of the arachidonic acid released during activation comes from phosphatidylcholine, the exposure of cells to dexamethasone must be causing the inhibition of both PLA_2 and phospholipase C pathways.

Experiments are continuing to use monoclonal antibodies to define receptors and molecules on the basophil surface involved in cell activation and secretion. Four monoclonal antibodies have been isolated which inhibit the binding of IgE to its receptor on the cell surface. Three of these antibodies bind competitively with IgE; the fourth monoclonal antibody (AA4) inhibits IgE binding but is not inhibited by IgE. The 3 hybridomas induce the release of histamine from both normal mast cells and rat basophilic leukemia cells. The fourth does not release histamine but can modulate the release reaction. In binding assays with intact immunoglobulin the three monoclonal antibodies bound to the same number of sites as IgE on the cell surface. In contrast, the fourth mAb binds to ~ 10-fold greater sites/per cell. Fab fragments of the four mAb were used to determine the number of binding sites on cells in comparison to IgE receptor number. One of the three monoclonal antibodies bound ~ 2.0 times the number of IgE receptor sites. Therefore, two molecules bind to each IgE receptor molecule which indicates an axis of symmetry in the receptor. The three hybridomas immunoprecipitate a broad band of protein with molecular weight of 50-55kDa from ^{125}I-surface labeled RBL-2H3 cells. The fourth antibody (AA4) is less efficient in precipitating of this band. The three antibodies did not react by immunoblotting to solubilized RBL-2H3 cells; however, the fourth (AA4) bound to a broad band of 14-16kDa as well as better defined bands of 30kDa, 35kDa and 55kDa. Immune precipitation of intrinsically labeled RBL-2H3 cells with the AA4 antibody precipitated several bands including those seen by immunoblots. The data support the conclusion that these three antibodies bind to the α component of the IgE receptor. The fourth antibody (AA4) however, seems to bind to a molecule closely associated with the IgE receptor and is important for modulating the release of histamine. Attempts are progressing to purify these molecules and characterize them further.

The binding of IgE to receptors on mast cells and basophils is a primary event in immediate hypersensitivity. When two adjacent IgE molecules are bridged, the cell is triggered to release histamine and other vasoactive mediators of inflammation. Monoclonal antibodies were used to define sites on the IgE molecule which are unavailable when the IgE is bound to receptors on the basophil. Eleven monoclonal antibodies were produced which bound to human IgE. They are all of the IgG class and by immunoblotting bind predominantly to the heavy, ε chain. Competitive binding assay demonstrates that these monoclonals recognize distinct epitopes on IgE, these sites are in 3 related groupings on the molecule. Four of 11 antibodies do not release histamine from washed human leukocytes. Six of the monoclonals were radiolabeled and used for binding to IgE, either on a solid matrix or to IgE-sensitized basophil preparations enriched by elutriation. All of the monoclonal antibodies bind well to IgE in the solid phase radioimmunoassay. Of the 6 labeled antibodies, the 3 that release histamine bind well to the basophil suspension. In contrast, the 3 monoclonals that did not release histamine bind very poorly to basophils i.e approximately 20-fold less than the histamine-releasing antibodies. Therefore, these monoclonal antibodies define sites on the IgE molecule that are either hidden or modified when IgE is bound to the Fc$_{\varepsilon}$ receptor on basophils. The characterization of these sites on the IgE molecule will define sites that are critical for binding to the receptor.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00007-24 LMI

PERIOD COVERED

October 1, 1984 to september 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Regulation of Carbohydrate Metabolism in Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wittenberger Charles L.	Chief, Microbiology Section	LMI, NIDR
Schoen Roberta	Microbiologist	LMI, NIDR
Abbe Kazuhiko	Visiting Fellow	LMI, NIDR
Abbe Shoko	Guest Worker	LMI, NIDR

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.80	2.80	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This investigation seeks to delineate the biochemical reactions involved in the inactivation and proteolytic degradation of a cell-associated fructosyltransferase (FTase) produced by Streptococcus salivarius. We continue to concentrate on the FT inactivation step because this reaction appears to 'mark' the enzyme for subsequent proteolysis FT when resting cell suspensions were incubated with low concentrations (0.5 μM) of copper ions, FT was inactivated in a time dependent manner. The activity of a cell-free preparation of the enzyme was unaffected by 5.0 μM cu^{++H}. Inactivation of the cell-associated FT required the catabolism of endogenous intracellular polysaccharide or exogenously supplied glucose and was prevented by inhibitors of glycolysis (iodoacetate, NaF, and Na_2HAsO_4). In starved permeablized cells, NADH could replace glucose whereas NADPH or ATP could not. The rate of FT inactivation under these conditions was markedly stimulated by O_2 while several other enzymes (glutamate dehydrogenase, phosphofructokinase, aldolase) were completely stable. Aerobically grown cells had high levels of NADH oxidase and low levels of FT, while cells grown under microaerophilic conditions exhibited the reverse relationship. Results from oxygen radical scavenger studies implicated $OH^{\cdot}$ in the inactivation. $OH^{\cdot}$ is thought to be formed as follows: 1) $FT\text{-}Cu^{++} + O_2^{\cdot -} \rightarrow FT\text{-}Cu^{+} + O_2$ 2) $FT\text{-}Cu^{+} + H_2O_2 \rightarrow FT\text{-}Cu^{++} + OH^{\cdot} + OH^{-}$. H_2O_2 and $O_2^{\cdot -}$ appear to be products of the NADH oxidase system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE-00022-17 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The role of cell envelope-associated molecules in the metabolism and ecology of oral bacteria.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

London Jack	Microbiologist	LMI, NIDR
Hausman, Sally	Microbiologist	LMI, NIDR
Kolenbrander, Paul	Microbiologist	LMI, NIDR
Weiss Ervin	Visiting Fellow	LMI, NIDR
Thompson, John	Visiting Scientist	LMI, NIDR

COOPERATING UNITS *(if any)*
Dr. Harold Neimark Downstate Medical Center, SUNY, New York, N.Y.
Dr. Roger Celesk Miles Laboratories, New Have, Conn.
Dr. Angelika Kagermeier Poliklinik for Zahnerhaltung, Erlangen West Germany

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35	1.35	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A study of the export systems of the xylitol and 2-deoxyglucose futile cycles has focused on the operation and regulation of a phosphohydrolase that cleaves xylitol-5-P and 2 deoxyglucose-6-P, respectively, prior to the expulsion of the free sugars. The enzyme hydrolyses a number of sugar phosphates, but is specific for (1) substrates phosphorylated at the terminal carbon position, (2) substrates with a D-erythro configuration and, (3) preferentially hydrolyzes those substrates that exist in a cyclical (pyranose or furanose) configuration. Enzyme activity is regulated by a membrane-associated component which can be removed from the membrane by extensive washing in high salt solutions. This regulatory mechanism accounts for the conservation of phosphorylated intermediates during the operation of the futile cycles and, more importantly, may be responsible for maintaining a balance between the intracellular levels of Pi and phosphorylated glycolytic intermediates under all conditions of growth.

The lectin-like protein responsible for coaggregration between Capnocytophaga gingivalis (emended) and Actinomyces israelii that is found on the cell envelope of the former has been identified and partially characterized. Two dimensional electrophoresis and western blot analysis used in conjunction with specifically adsorbed antisera revealed that the lectin is a basic protein (pI=8.4) with a molecular weight of 155KDa. The protein is located exclusively on the outer member of C. gingivalis.

Progress on the identification characterization of lectins found on cells of Bacteroides loeschei 1295 and Capnocytophaga ochracea stain 25 that are responsible for coaggregation with strains of Streptococcus sanguis and Actinomyces israelii will be summarized in Dr. Paul Kolenbrander's report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00034-17 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Histamine Release

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	NIDR LMI
Hook, William A.	Research Microbiologist	NIDR LMI
Stracke, Mary L.	Medical Staff Fellow	NIDR LMI
Basciano, Luann K.	Microbiologist	NIDR LMI
Berenstein, Elsa H.	Microbiologist	NIDR LMI
Garcia-Gil, Mercedes	Visiting Fellow	NIDR LMI
Essani, Naeen A.	Visiting Fellow	NIDR LMI
Takaishi, Toshiaki	Visiting Fellow	NIDR LMI

COOPERATING UNITS *(if any)*

NIAMDD, Arthritis & Rheumatism Branch, NIH; NHLBI, Laboratory of Chemical Pharmacology, NIH (M. Beaven): Walter Reed Army Institute of Research (P. Chiang); NIAID, LCI, NIH; NICHD, ODCPR, NIH (M. Karten).

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.00	5.00	2.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody, the anaphylatoxins, LHRH peptides, and the Ca^{2+} ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and biochemical changes during cell activation. Large numbers of cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01- DE-00042-15 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biological Characterization of Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chassy Bruce M.	Research Chemist	LMI, NIDR
Porter Emily V.	Chemist	LMI, NIDR
Sharer susan	Microbiologist	LMI, NIDR
Flichinger Jeannette	Microbiologist	LMI, NIDR
Hull Eunice	Bio Lab Technician	LMI, NIDR

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.45	3.45	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to use genetic, biochemical and physiological approaches to investigate the pathogenicity of oral bacteria. The three specific areas of investigation are: 1) molecular cloning and characterization of genes encoding surface structures that mediate attachment of oral microbes, 2) characterization of plasmid-coded and chromosomal metabolic genes from oral bacteria and 3) development of systems for genetic exchange in oral bacteria. A gene encoding the structural subunit of the Type 2 fimbriae of Actinomyces viscosus was cloned into Escherichia coli using the cosmid vector pHC79. This has was subcloned into pUC13 on a 10 Kbp HIND III fragment; the clone expresses the complete 59 Kdal fimbrial subunit. Subcloning for M13 dideoxy sequencing in now in progress. A gene encoding the synthesis of the Type 1 fimbriae was identified in a second genomic library; its sequence is under study. The gene for the Type 2 fimbriae of A. naeslundii was also cloned; it was found to share DNA-DNA homology with the gene isolated from A. viscosus. An unusual plasmid encoding the enzyme β-galactosidase was found in L. actobacillus casei; the structural gene was cloned into E. coli using the plasmid expression vector pKK223-3. Subclones are being prepared for sequencing and for analysis of L. casei promoter structure and function. For the first time Lactobacillus strains were transformed; using lactose plasmids encapsulated in liposomes prepared from lecithin, lactose fermenting transformants were derived from lactose negative strains of L. casei. Southern blot anaylsis confirmed the introduction of specific lactose metabolic genes into the L. casei chromosome. Current studies seek to optimize and improve the transformation system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00043-15 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Genetic Studies on Pathogenic Oral Microorganisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Donkersloot Jacob A.	Research Microbiologist	LMI, NIDR
Harr Robert J.	Bio Lab. Tech. (Micro.)	LMI, NIDR
Hull Eunice M.	Bio. Lab. Tech. (Micro.)	LMI, NIDR

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.70	1.00	1.70

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cosmid gene library of Actinomyces viscosus T14V was prepared in Escherichia coli to examine the expression of A. viscosus antigens and to gain insight into the structure of A. viscosus type 1 and type 2 fimbriae. Out of this library of 550 clones, 28 reacted in a colony immunoassay with antibodies against A. viscosus cells. The proteins responsible for these reactions were identified in 3 clones. Clones AV1209 and AV2009 displayed nonfimbrial antigens with subunits of 40 and 58 kilodaltons, respectively. Clone AV1402 showed a 59-kilodalton protein that reacted with monospecific antibody against type 2 fimbriae and that comigrated with a subunit of type 2 fimbriae during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This indicates that AV1402 expresses a gene (fimA) for a subunit of A. viscosus type 2 fimbriae.

Mapping and shotgun subcloning experiments with BamHI showed that fimA was contained within the 9.7-kb HindIII B fragment of pAV1402. This fragment was subcloned into pUC13. Expression of p59 was strongly orientation dependent, which indicates that the Actinomyces promoter for fimA has little or no activity in E. coli. Further mapping revealed that fimA expression in AV1402 was depende upon the nearby P2 promoter of tet in the vector.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-046-13 LMI

PERIOD COVERED

October 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chronic Inflammation and Immunomodulation of Connective Tissue Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wahl, Sharon	Chief, Cellular Immunology Section	LMI, NIDR
Ohura, Kiyoshi	Visiting Fellow	LMI, NIDR
Evequoz, Vlasta	Visiting Fellow	LMI, NIDR
Hunt, Denise	Microbiologist	LMI, NIDR
Dougherty, Suanne	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. I. Katona, Naval Medical Research Institute
Dr. R. Wilder, ARB, NIADDK   Dr. D. Chenoweth, VA Medical Center, San Diego, CA
Dr. Yocum, ARB, NIADDK   Dr. A. Hand, LBS, NIDR

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.35	1.70	2.65

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mononuclear cell recruitment and activation are central to the initiation, augmentation and perpetuation of chronic inflammatory lesions. Investigations in this laboratory continue to examine mechanisms of monocyte directed migration (chemotaxis) toward inflammatory stimuli. In order to study simultaneous cell surface chemotactic receptor expression on individual cells, two different color fluorescent chemotactic ligand probes, FITC-C5a and TR-FMLP, were developed and characterized. The majority of peripheral blood monocytes were found to co-express C5a and FMLP chemotactic receptors as determined by flow microflorometry. In contrast, monocytes obtained from chronically inflamed synovium of rheumatoid arthritis patients did not migrate to C5a nor did they express C5a receptors, whereas FMLP receptors and chemotaxis to FMLP were normal. This differential regulation of chemotactic ligand receptor expression and function also occurs following in vitro monocyte activation and may provide insight into mechanisms of chemotaxis abnormalities in certain diseases. Not only are synovial inflammatory cells abnormal in their response to chemotactic stimuli, but recent studies also demonstrate abnormal production of a chemoattractant by synovial inflammatory T lymphocytes. Synovia from anergic arthritis patients which have a heavy mononuclear cell infiltrate produce excess amounts of lymphocyte derived chemotactic factor (LDCF), whereas synovia from nonanergic patients which are relatively hypocellular do not produce LDCF. Thus, the production of LDCF by anergic synovia may provide a critical stimulus in the continual mononuclear cell infiltrate seen in these patients. Once mononuclear cells are recruited to the inflamed synovium they generate other cytokines which enhance and perpetuate the inflammatory lesion. One of these cytokines, fibroblast activating factor (FAF), stimulates synovial fibroplasia leading to excessive collagenase and prostaglandin synthesis involved in joint destruction. Because of its potential key involvement in the pathogenesis of rheumatoid synovial lesions, efforts are being directed at characterizing and cloning FAF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00061-09 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complement Activation and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandberg, Ann L.	Chief, Humoral Immunity Section	LMI, NIDR
Cisar, John	Research Microbiologist	LMI, NIDR
Mudrick, Lewis Linda	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.80	8.0	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several aspects of the interaction of the complement system with Actinomyces viscosus T14V and A. naeslundii WVU45, organisms implicated in the pathogenesis of periodontal disease, are under exploration. Complement fixation by monoclonal antibodies has been used to localize epitopes on the fimbriae of these bacteria. These studies have provided evidence that the subunit structure of the type 1 fimbriae (which mediate attachment of A. viscosus T14V to saliva treated hydroxyapatite) and the type 2 fimbriae (which are associated with lectin activity for saccharide receptors on bacteria and mammalian cells) differ. Thus, each of three monoclonal antibodies specific for the type 1 fimbriae in the absence of a second antibody activates the complement sequence. In contrast, only one of four monoclonal antibodies specific for the type 2 fimbriae (all of which consume complement when assayed in combination with a second antibody) activates this mediator system. Since complement activation by IgG antibodies is strictly dependent on the bridging of two antibody molecules by C1q within a given distance, the type 2 fimbriae are apparently more extended than the type 1 fimbriae, a finding which has been suggested by recent electron microscopic examination of the fimbriae. The biological activity of one monoclonal antibody can clearly be influenced by the addition of a second antibody. Combinations of monoclonal antibodies has revealed inhibition, enhancement, addition and synergy of complement activation. Investigation of complement and non-complement dependent destruction of the actinomyces by polymorphonuclear leukocytes has revealed multiple mechanisms. The lectin associated with the type 2 fimbriae can initiate phagocytosis by attaching to carbohydrate containing receptors exposed by sialidase treatment of PMNs. This interaction is abolished by the addition of lactose. Antibodies reactive with the cell wall or the two types of fimbriae are effective opsonins for the induction of Fc receptor mediated phagocytosis. In addition, C3b/C3bi deposited on the bacterial cell wall by antibody initiated activation of the complement cascade enhances antibody mediated phagocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00216-09

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological Control of Connective Tissue Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wahl, Larry M.	Research Biologist	LMI, NIDR
Wahl, Sharon M.	Chief, Cellular Immunology	LMI, NIDR
Lampel Leroy L.	Bio. Lab. Technician	LMI, NIDR

COOPERATING UNITS *(if any)*

David S. Finbloom, Department of Immunology, Walter Reed Army Institute of Res.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.05	1.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the mechanisms of monocyte/macrophage activation and resultant production of biochemical mediators continue with the goal of relating these events to the regulation of connective tissue metabolism and immune dysfunction in various disease states. Previous findings utilizing guinea pig peritoneal macrophages revealed that collagenase production was inhibited by indomethacin, dexamethasone or colchicine. In contrast to collagenase production our recent studies demonstrate that another macrophage product, fibroblast activating factor (FAF), which enhances collagen synthesis was relatively unaffected by these same anti-inflammatory agents. These results suggest differential regulation of the pathway for destruction and formation of connective tissue. We have also extended these studies in the human system. Con A stimulates human monocytes to release significant amounts of collagenase through a prostaglandin dependent pathway. Whereas dexamethasone inhibits monocyte collagenase at the PGE_2 level, colchicine inhibited collagenase synthesis without effect on PGE_2 synthesis. In contrast, the inhibition of collagenase by retinoic acid was accompanied by a significant increase in PGE_2 synthesis. Since neither the inhibition by colchicine or retinoic acid could be reversed by PGE_2, these studies provide insight into the intracellular events leading to monocyte collagenase synthesis and secretion and also provide information on the mechanism of action of certain inflammatory drugs. Interferon-" (IFN-") which is a potent immunomodulator of many macrophage functions has been shown to bind to purified human monocytes as well as monocyte like cell lines (U937 and HL60. Radioiodinated recombinant IFN-" bound to human monocytes and the cell lines in a specific and saturable manner. Saturation of binding sites occurred at 10^{-9} M and there were 4,000 specific binding sites per cell for both monocytes and cell lines. Purified lymphocyte derived IFN-" as well as recombinant rIFN-" competed for binding sites with ^{125}I-rIFN-". Additionally, there was no inhibition of binding of the ^{125}I-rIFN-" by rIFN-^{3} or rIFN-^{2}.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00254-08 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microbial Antigens Associated with Specific Adherence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cisar John	Research Microbiologist	LMI, NIDR
Yeung Maria K.	Post-Doctoral Fellow	LMI, NIDR
Hurst-Calderone Susan L.	Microbiologist	LMI, NIDR
Sandberg Ann	Chief, Humoral Immunity	LMI, NIDR
Mergenhagen Stephan E.	Chief, Microbiology and Immunology	LMI, NIDR

COOPERATING UNITS *(if any)*

W. B. Clark,	University of Florida,
F. C. McIntire	University of Colorado
A. E. Vatter	University of Colorado

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.70	1.30	2.40

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

 ☐ (a1) Minors

 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are continuing to define the fimbrial adhesins of Actinomyces viscosus, an oral bacterium that colonizes tooth surfaces, forms dental plaque and contributes to the initiation of gingivitis and periodontal diseases. The type 1 fimbriae of actinomyces appear to be a principal adhesin for tooth surfaces as shown by studies of bacterial adsorption to saliva-treated hydroxyapatite. Recombinant DNA technology has been used to clone a structural gene that encodes the type 1 fimbrial subunit of A. viscosus strain T14V. This protein has a molecular weight of 62,000 daltons and is reactive with monospecific as well as monoclonal antibodies directed against actinomyces type 1 fimbriae. The type 2 fimbriae of actinomyces are antigenically and functionally distinct from type 1. Type 2 fimbriae account for the lactose-sensitive coaggregation of actinomyces with strains of Streptococcus sanguis and thus would be expected to promote interbacterial adherence within dental plaque. The gene encoding the type 2 fimbrial subunit of strain T14V has been cloned and the protein expressed in Escherichia coli has been partially purified. In addition to the study of type 2 fimbriae, immunochemical studies are continuing to define streptococcal carbohydrates that function as receptors for the actinomyces lectin. The receptor-associated polysaccharide of S. sanguis 34 has been purified and found to have N-acetylgalactosamine as the immunodominant group. These findings have begun to provide a structural basis for understanding the mechanisms of oral microbial adherence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE-00273-07 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell-Cell Interactions Between Oral Actinomyces and Other Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kolenbrander, Paul	Research Microbiologist	LMI, NIDR
London, Hack	Research Microbiologist	LMI, NIDR
Ciardi, Joseph	Research Microbiologist	LMI, NIDR
Andersen, Roxanna	Microbiologist	LMI, NIDR
Tylenda, Carolyn	Research Microbiologist	LMI, NIDR
Weiss, Ervin	Visiting Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*
Dr. A.L. Delisle, University of Maryland, School of Dentistry, Baltimore
Dr. L.V. Holdeman, VPI and SU Blacksburg, VA
Dr. A. Tylenda, Georgetown University, School of Dentistry

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.20	3.00	1.20

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As additional information regarding cell-to-cell recognitions among oral bacteria is discovered, it is becoming increasing clear that a dynamic but organized microbial community exists in the oral cavity. The part of this complex community studied in this laboratory pertains especially to microbial ecology. Many of the bacteria that commonly inhabit this region were tested by an in vitro simulated plaque model. In the presence of potential competitors, several strains were tested as examples to support certain principles of coaggregation. Results of this study indicated that the oral cavity is replete with competitors and cohorts. Strains that belonged to different genera could recognize common third partners and prevented each other from coaggregating with them. Alternatively, demonstrations of multigeneric bacterial complexes were commonplace, indicating the similarity to associations of bacteria found in dental plaque.

The surface structures that mediate these coaggregations were studied by using bacteriophage as surface probes and by isolating coaggregation-defective mutants. Identity of one receptor with a mediator of coaggregation was shown by altered coaggregation properties of bacteriophage-resistant mutants compared to their parent strains. To aid in further study of these surface structures, eight new actinomyces phage were isolated for the first time from human dental plaque. Studies with coaggregation-defective mutants revealed that certain outer membrane or surface proteins were altered or missing in the mutants but present in the parent strain.

Results of a survey of the coaggregation properties of nearly 100 strains of Fusobacterium, Selenomonas, and Veillonella indicated that fusobacteria were very reactive, selenomonas hardly recognized any partners, and veillonella exhibited many lactose-inhibitable coaggregations. The results of each of these investigative approaches are focused on understanding the relationship of cell surface recognitions among oral bacteria and their role in microbial ecology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00290-6 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Production of Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	NIDR LMI
Hook, William A.	Research Microbiologist	NIDR LMI
Basciano, Luann K.	Microbiologist	NIDR LMI
Berenstein, Elsa H.	Microbiologist	NIDR LMI
Fischler, Cynthia	Medical Technician Micro.	NIDR LMI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.30	1.00	3.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the Fc_ϵ receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00341-03 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of sugar transport and metabolism in lactic acid bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thompson, John	Visiting Scientist	LMI, NIDR

COOPERATING UNITS *(if any)* Michael A. Curtis, LMI, NIDR, Bruce M. Chassy, LMI, NIDR Jack London, LMI, NIDR, Sally Z. Hausman, LMI, NIDR Dennis A. Torchia, Mineralized Tissue Research Branch, NIDR William Egar, Division of Biochemistry and Biophysics, Office of Biological Research and Review, FDA.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	.80	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A membrane-regulated, sugar-phosphate: phosphohydrolase has been purified and characterized from Lactobacillus casei. Antibodies to the enzyme have been obtained for use in structure/function experiments. Lactose analogs containing non-metabolizable, $[^{14}C]$-labeled 2-fluoro-D-glucose or 2-deoxy-D-glucose have been prepared enzymatically. Mutants of Streptococcus lactis have been isolated by positive resistance and UV mutagenesis procedures. These mutants lack ATP-dependent glucokinase and/or PEP-mannose: phosphotransferase activities and are incapable of growth on glucose. Through the use of the bacterial mutants, and transport studies with $[^{14}C]$ 2F-lactose and $[^{14}C]$ 2D-lactose, definitive evidence for intracellular phosphorylation of free sugars via the PTS has been obtained. Biochemical, and physiological studies with Peptostreptococcus anaerobius have revealed that proline is a requirement for the transport and metabolism of glucose by this anaerobic oral pathogen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00374-04 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Generation of GM-CSF by Short-Term Cultures of Murine Bone Marrow

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Pluznik, Dov H.	Visiting Scientist	LMI	NIDR
Tsuda, Hiroyuki	Visiting Fellow	LMI	NIDR
Weedon, Lynda	Bio Lab Tech	LMI	NIDR
Mergenhagen, Stephan E.	Chief, LMI, NIDR	LMI	NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Granulocytes and Macrophages (GM) are important cellular components in inflammation. The bone marrow (BM) contains stem cells which under the control of the lymphokines designated colony stimulating factors (CSF) proliferate and differentiate into mature GM. In a previous report we showed that murine BM cells when stimulated simultaneously with tumor promoting phorbol esters (TPA) and bacterial lipopolysaccharides (LPS) produce their own CSF. In the present studies we characterized the BM cells which responded to TPA and LPS in generating CSF and also identified the subclasses of CSF produced under these conditions. CSF was quantitated by measuring ^{3}H-TdR incorporation into the DNA of a CSF dependent basophil/mast cell line PT-18. Only BM cells adherent to polystyrene coated with goat anti mouse immunoglobulin (Ig) antibodies generated CSF suggesting that cells with receptors for Ig (B-lymphocytes) and/or Fc (GM) are involved in generation of CSF. We next identified the subclasses of CSF produced by the BM (M-CSF, G-CSF, GM-CSF and multi-CSF). M-CSF was excluded since the BM-CSF was sensitive to trypsin digestion while M-CSF is resistant. Multi-CSF was excluded since DA-1 cells, which respond to multi-CSF but not to GM-CSF, did not respond to BM-CSF. BM-CSF induced differentiation in M1 myeloid leukemic cells which respond only to G-CSF. Thus, BM-CSF contains GM-CSF and G-CSF. Receptor triggering by agonist is followed by inositol phospholipid breakdown to diacylglycerol (DAG) and inositol triphosphate (InsP3). DAG activates protein kinase-C (PK-C) and it can be replaced by TPA. InsP3 is responsible for Ca^{+2} mobilization and can be replaced by Ca^{+2} ionophores. To determine whether generation of BM-CSF requires activation of PK-C and Ca^{+2} mobilization we tested the ability of A23187 (Ca^{+2} ionophore) to replace LPS and DAG to replace TPA. A23187 and TPA acted cooperatively to stimulate the generation of CSF. Trimethoxybenzoate (TMB-8) an inhibitor of Ca^{+2} mobilization inhibited stimulation of CSF production either by TPA and LPS or by TPA and A23187. DAG could replace TPA in cooperating with A23187 to stimulate the generation of CSF. Thus, activation of PK-C and Ca^{+2} mobilization are probably early signals in the generation of CSF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00381-02 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Mechanisms of Colonization of Oral Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Ciardi, Joseph E.	Research Biochemist	NIDR LMI
Kousvelari, Eleni	Senior Staff Fellow	NIDR CIPCB
Kolenbrander, Paul E.	Research Microbiologist	NIDR LMI
Mull, Colette C.	COOP Student	NIDR LMI
Thornton, Angela	Federal Junior Fellow	NIDR CIPCB

COOPERATING UNITS (if any)

University of Oslo, Norway

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.56	1.03	0.53

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Adsorption of bacteria to saliva-coated surfaces, saliva-induced aggregation of bacteria and interactions between different genera of oral bacteria (coaggregation, interbacterial adherence) are considered important in the formation and persistence of dental plaque.

Studies have been carried out to define the galactoside specificity in the lectin reaction specific for the coaggregation between Streptococcus sanguis and Propionibacterium acnes. Physiochemical interactions important for this coaggregation have also been defined. Experiments to isolate and/or to determine stability of cell surface coaggregation factors have yielded results consistent with a sensitive lectin-protein on the S. sanguis and a stable carbohydrate-containing receptor on the P. acnes.

Growth of Actinomyces viscosus and Streptococcus sanguis strains in sublethal concentrations of antibiotics has resulted in the production of variants with altered ability to undergo coaggregations and/or to adsorb to saliva-coated hydroxyapatite. Studies are in progress to characterize antibiotic induced surface-structure changes at the molecular level.

Parotid salivas from rats treated with β-adrenergic antagonist or β-adrenergic agonist show different potentials to support bacterial aggregation and adsorption of bacteria to hydroxyapatite. These differences have been associated with biochemical changes in saliva composition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00382-02

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Growth and Interaction of Oral Microorganisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robrish, Stanley A.	Research Microbiologist	NIDR LMI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pure and mixed cultures of the coaggregating pair; Fusobacterium nucleatum and Streptococcus sanguis (CC5A) have been grown successfully in both the batch and continuous modes. Pure culture studies revealed that F. nucleatum used glutamic acid and glucose concurently during exponential growth in batch culture while less than half the glucose, and all of the glutamic acid were used in continuous culture. Glutamic acid seemed to be used in preference to glucose by F. nucleatum. Lysine was used after glucose and glutamic acid during batch growth of F. nucleatum.

A stable mixed culture, including extensive coaggregation of these organisms was obtained when CC5A was inoculated into the continuous culture medium before F. nucleatum. In contrast, using the same conditions, the growth of CC5A was suppressed if F. nucleatum was inoculated first. When growth together in continuous culture, F. nucleatum was reduced to very low levels while CC5A became dominant as the dilution rate was raised. F. nucleatum remained in the culture and its increase was reflected by an increased butyric acid production after the dilution rate of the culture was reduced.

Butyric acid production proved directly related to the growth rate of F. nucleatum. In pure culture, two rates of cell growth and butyric acid formation were seen, while in a batch grown mixed culture, only a single rate of butyric acid production was observed. The interactions of the two organisms during butyrate production are unknown and under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE-00383-02 LMI

PERIOD COVERED
October 1, 1984-September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biogenesis of Methane in Dental Plaque

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Christopher W. Kemp	Laboratory Technician	LMI, NIDR
Michael A. Curtis	Visiting Scientist	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Twelve strains of oral methanogenic bacteria were isolated from supra- or subgingival plaque deposits by continuous culture techniques which yielded populations containing >50% methanogens. Taxonomic studies based on substrate utilization studies (e.g., $CO_2 + H_2$, formate and ehtanol), morphological studies (cell wall composition) and DNA homology studies suggested that all twelve isolates belong to the genus Methanobrevibacter. However, interspecies comparisons between Methanobrevibacter smithii, methanobrevibacter ruminantion and the twelve isolates revealed that the oral strains were not related (at the species level) to any of the known intestinal or rumen Methanobrevibacter species. Furthermore, comparisons of DNA homologies among the twelve isolates indicated that they could be assigned to three distinct toxous. Thus, this initial study suggests that, at least, three distinct species of methanogenic bacteria exist in the oral cavity.

Model food chain systems were demonstrated first in binary cultures in which H_2 and CO_2 were supplied to one of the methanogenic isolates by Veillonella rodentium growing at the expense of lactate in Peptostreptococcus anaerobius growing at the expense of glucose and various amino acids. Tripartite cultures consisting of saccharolytic bacterium (i.e., Streptococcus mutans, Streptococcus sanguis or Lactobacillus casei). V. rodentium and one of the methanogenic isolates provide a functional food chain model wherein sugar was converted to lactate for formate ethanol and acetate which was in turn converted into substrates for the metahnogens (i.e., H_2 and CO_2). In this model, however, formate also served as a source for methane production with some of the metanogenic bacteria. The fact that methane production in the tripartile cultures was not as great as that observed with plaque samples suggests that the natural system provides a more efficient hydrogen transfer chain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE- 00384-02 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of IgA1 protease on the biological activity of secretory IgA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cole Michael F.	Visiting Scientist	LMI, NIDR
Hsu Dana S.	Laboratory Technician	LMI, NIDR

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Humoral Immunity

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.10	1.10	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Forty aerobic and 20 anaerobic bacterial isolates obtained from monkey plaque have been screened to determine whether they produce an IgA1 protease specific for monkey IgA. While a number appear to possess proteases capable of hydrolyzing monkey IgA none, so far, appear capable of cleaving monkey IgA at the hinge region to produce Fab and Fc fragments.

Attempts to identify particular isolates have proved difficult as the monkey isolates seem not to fall readily into existing taxonomic schemes.

Monomeric IgA has been purified from a pool of normal monkey serum. Attempts to determine the distribution of the 1 and 2 subclasses in the purified monkey serum using monoclonal and monospecific antibodies to human IgA1 and 2 has not been successful because these reagents do not appear to cross-react with monkey IgA.

IgA1 protease from Streptococcus sanguis 10556 has been partially purified by high performance liquid chromatography.

Pregnant monkeys have been immunized with type 1 fimbriae of A. viscosus and type 2 fimbriae of A. naeslundii and A. viscosus in an attempt to produce colostral IgA and serum IgG antibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-0385-02 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lectin Dependent Adherence of Actinomyces to Human Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brennan, Michael	Staff Fellow	LMI, NIDR
Cisar, John	Microbiologist	LMI, NIDR
Sandberg Ann L.	Chief, Humoral Immunity Section	LMI, NIDR

COOPERATING UNITS *(if any)*

Al Vatter, Univ. of Colorado Medical Center

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.30	1.30	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the adherence of oral actinomyces to human epithelial (KB) cells have demonstrated that attachment is initiated by a fimbrial lectin on these bacteria which interacts with receptors exposed by sialidase, an enzyme secreted by actinomyces. Binding and inhibition studies indicate that the plant lectins from peanut and Bauhinia purpurea interact with the same receptor as the bacterial lectin. These lectins detect a band of 160 kd on sialidase-treated Western blots of KB cell extracts separated by SDS-PAGE. This 160 kd component has been partially purified by lectin affinity chromatography and has been identified as a membrane associated sialoprotein by a variety of analytical and radiolabelling techniques. The receptors for the actinomyces apparently contain GalB3GalNAc termini which are exposed after sialidase treatment of the 160 kd cell surface glycoprotein. Structural analysis of the 160 kd glycoprotein by biochemical and immunological techniques is in progress as is the production of monoclonal antibodies against this glycoprotein and the disaccharide GalB3GalNAc. Related studies on the interaction of human erythrocytes with actinomyces suggest that glycolipids rather than glycoproteins are the receptors for the actinomyces lectin on these mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00386-02 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Neuropeptides in Immune and Inflammatory Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ruff R. Michael	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. C. Pert, Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neuropeptides, small signal peptides largely known for their role as transmitters of nerve impulses in the brain which mediate mood and emotion, have now been shown to regulate immune system function. Our work reveals that human monocytes have receptors and will respond chemotactically to numerous neuropeptides. Neuropeptides which we have reported on include ^{2}-endorphin and other opiates, substance P and bombesin. We have shown that a major class of psychoactive drugs, the benzodiazepines, are also potent chemoattractants. In this case we have directly demonstrated the presence of chemotactic receptors through ligand binding experiments. The presence of multiple, diverse, neuropeptide chemotactic receptors on monocytes and other immune system cells suggests the existence of a neuroendocrine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

In addition to the presence of neuropeptide receptors we have also been able to demonstrate that human aveolar macrophages store and secrete the neuropeptide bombesin. Neuropeptide synthesis may therefore be a general feature of various immune cell populations. Although speculative, such results are consistant with the possibility of a multi-direc-tional communication network via neuropeptides and their receptors. The purpose of such a network would be to link the body's cellular defense and repair systems with the nervous and endocrine systems and thereby integrate the internal millien of the entire organism.

Additional work has suggested that a major cause of human cancer, small cell lung carcinoma, may not, as previously thought, arise from lung epithelium but originates from hemopoietic cells when the normal macrophage mediated repair of lung tissue is deranged by continouous heavy smoking. This work represents a major conceptual shift in the understanding of this disease as well as other cancers in which there exists an in flammatory component and suggests novel strategies for disease control with clinical implications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00391-02 LMI

PERIOD COVERED

October 1, 1984 to September 31, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sugar and amino acid metabolism in Peptostreptococcus anaerobius

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Curtis, Michael A.	Visiting Associate	LMI, NIDR
Thompson, John	Visiting Scientist	LMI, NIDR

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS: 1.10	PROFESSIONAL: 1.10	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is an examination of the role of proline in glucose metabolism by Peptostreptococcus anaerobius ATCC 27337. Resting cells of P. anaerobius were shown to have an absolute requirement for low concentrations of L-proline in order to utilize glucose. The requirement was shown to reside at the level of initial transport of the sugar into the cell: no accumulation of radiolabelled glucose occurs in resting cells until the addition to the reaction vessel of the imino acid. The action of proline on sugar transport is proposed to occur via the activation of the metabolism of an endogenous reserve of amino acids, notably alanine, within the cell. The reduction of proline to -amino valeric acid allows for the removal of reducing equivalents generated from the oxidation of the endogenous amino acid pool. The metabolism of these amino acids gives rise to the formation of a phosphoryl donor for the sugar phosphotransferase system which can then transport into the cell the extracellular sugar. These studies have thereby demonstrated a unique interelationship of sugar transport and amino acid metabolism. That each of these processes is dependent on an exogenous source of proline reflects the relationship that exists between the oral peptostreptococci and the high proline rich proteins of their natural habitat.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00392-02 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of HTLV-III and CMV on Monocyte/Macrophage Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Smith, Phillip	Senior Staff Fellow	LMI, NIDR
Wahl, Sharon	Chief, Cellular Immunology	LMI, NIDR
Wahl, Larry	Microbiologist	LMI, NIDR
Midura, Sharon	Microbiologist	LMI, NIDR
Miyake, Yoichiro	Visiting Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*

Thomas Folks	Howard E. Genedlman, M.D.	LIR NIAID
Alain H. Rook	H. Clifford Lane	LIR NIAID
Anthony S. Fauci, MD - LIR, NIAID	Ildy Katona, MD	USUHS (Navy)

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.30	1.15	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two important viruses, human lymphotropic leukemia virus III (HTLV-III) which causes the acquired immune deficiency syndrome and cytomegalovirus (CMV) which is responsible for cmv disease which is responsible for CMV disease induce immunosuppression in vitro and in vivo. However, which causes the acquired immune deficiency syndrome the role of monocytes/macrophages in this immunosuppression is unknown. Therefore, the goal of this project has been to determine whether these viruses can infect human monocytes and whether infected monocytes exhibit impaired effector cell function and/or antigen-presentation. The results obtained thus far indicate that both HTLV-III and CMV can infect human monocytes. Furthermore, viral-infected monocytes appear to have impaired cell functions including cytotoxicity, chemotaxis, inflammatory mediator (interleukin-1, prostaglandin E_2) production and antigen-presentation. This information indicates that the monocyte/macrophage likely contributes to the in vivo immunosuppression caused by HTLV-III and CMV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE-00397-01 LMI

PERIOD COVERED

December 1, 1984 to September 3o, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Fibroblast Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Agelli, Maria	Visiting Fellow	LMI, NIDR
Wahl, Sharon	Chief, Cellular Immunology Section	LMI, NIDR
Tsuda, Hiro	Visiting Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. M. Sobel	NCI
Dr. M. Young	MTBR, NIDR

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.60	.75	.85

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inflammatory cell regulation of fibroblast growth and function may be instrumental in normal wound repair and healing. However, in chronic inflammation the prolonged release of inflammatory mediators can lead to pathologic changes in connective tissue. Therefore, investigations in this laboratory focus on the production and mechanism of action of a T cell product which can regulate fibroblast growth and function. Human peripheral blood T lymphocytes are activated by concanavalin A and other mitogens to produce fibroblast activating factor (FAF), a mediator that stimulates proliferation of dermal fibroblasts. FAF has been purified at least 5,000-fold using anion-exchange chromatography, gel filtration and preparative PAGE electrophoresis. A leukemia T-cell line, HSB_2, produces FAF when stimulated with mitogens and can potentially be used to produce large quantities of FAF for biochemical purification, as well as for molecular cloning of the FAF gene.

ANNUAL REPORT OF THE LABORATORY OF ORAL BIOLOGY AND PHYSIOLOGY

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Laboratory of Oral Biology and Physiology conducts basic research on the structure and function of secretory cells and tissues, and on specific enzyme-catalyzed posttranslational protein modifications. The Laboratory is divided into two research groups: the Experimental Morphology Section, which employs structural, biochemical and immunological approaches to investigate the secretory process in the salivary glands and pancreas; and the Enzyme Chemistry Section, which focuses on the mechanisms of action and physiological significance of the transglutaminases and their products, and the formation, fate and function of the amino acid hypusine and the protein in which it occurs. Steady progress has been made during the past year on all ongoing research projects. Highlights of the laboratory's efforts are presented below.

EXPERIMENTAL MORPHOLOGY SECTION

Cyclic AMP-mediated intracellular events play major roles in the differentiation and function of rat parotid secretory cells. These phenomena are being studied in a number of experimental systems which include acute and chronic β-adrenergic stimulation, in vitro incubations with various secretagogues, and exposure of animals to altered gravity conditions. Although the extent of the response varies, changes in the intracellular distribution of the regulatory subunits of cyclic AMP-dependent protein kinase are consistently observed. Changes in phosphorylative activity and in the substrate proteins for the kinase also occur. These findings suggest that compartmentalization of certain regulatory proteins, and alterations in their compartmental distribution, may represent significant control mechanisms for the expression of their activity.

The lingual serous (von Ebner's) gland of the tongue synthesizes and secretes the digestive enzymes lingual lipase and amylase. Purification and characterization of these enzymes is currently underway, and the regulation of their secretion is being studied using in vitro and in vivo systems. Enzyme secretion is mainly regulated by the cholinergic system; in contrast to the parotid gland, β-adrenergic stimulation results in low rates of secretion. Additionally, an apparent non-parallel release of the two enzymes occurs with β-adrenergic stimulation.

In addition to the secretion of proteins, fluid, and electrolytes, exocrine glands also take up a variety of substances from the surrounding milieu. Studies conducted in LOBP over the past several years have been directed toward characterizing the endocytosis of exogenous proteins by the acinar and duct cells. Utilizing ferritin as an ultrastructural tracer, a system of apical tubules and vesicles involved in protein uptake from the lumen has been identified in striated duct cells of the rat parotid gland. Its function appears to be complementary to that of the large apical vacuoles previously described in the duct cells, and together they make up an efficient system for clearing certain foreign proteins from the lumen. Although evidence exists suggesting that this endocytic system is involved in salivary protein uptake in certain pathological conditions, it remains to be determined whether or not it also functions to take up salivary proteins in the normal gland.

Efforts have continued to further refine the culture system for exocrine acinar cells described in last year's report. Provision of a suitable substrate for cell attachment, and use of a highly supplemented medium allows for growth and proliferation of differentiated acinar cells. Culture dishes coated with basement membrane extract give the best results, but attachment and growth also occur in Primaria dishes and in standard dishes coated with laminin. These cultures now provide an opportunity to conduct a variety of studies on exocrine cell growth, differentiation and function not possible under in vivo conditions.

ENZYME CHEMISTRY SECTION

Considerable progress has been made in studies of hypusine formation and the structure of the protein in which it occurs, eIF-4D. The enzyme which catalyzes the oxidation of protein-bound deoxyhypusine to hypusine has been partially purified. A specific assay for the enzyme has been developed, along with several other useful assays for it. Characterization of the reaction has demonstrated that the deoxyhypusine hydroxylase is not the same as the lysyl- and prolylhydroxylases acting on collagen, and that it does not require ascorbic acid or α-ketoglutarate for activity.

The amino acid sequence of the region of eIF-4D in which hypusine occurs has now been determined. A nucleotide probe for a portion of this sequence has been prepared, and will be used for identification and cloning of the gene for eIF-4D. Antibodies to eIF-4D, as well as to the peptide containing hypusine, are currently being prepared. These reagents should provide the basis for experiments to determine the biological function and significance of eIF-4D.

The transglutaminases continue to be a major resarch emphasis of the Enzyme Chemistry Section. Studies conducted during the past year have focused on the association of transglutaminase activity with tissue matrix elements, and the modulation of factor XIIIa activity and fibrin stabilization by albumin and other physiological compounds. The lung contains the highest level of transglutaminase activity of any organ, and most of this activity is associated with insoluble matrix elements rich in elastin and proteoglycan. This transglutaminase can be dissociated from the matrix in the presence of sulfhydryls, with a concomitant 40-fold increase in activity. In contrast to the whole organ, cells derived from the lung contain predominately cytosolic enzyme, suggesting that the matrix-associated transglutaminase may play a role in tissue organization and assembly. Preliminary studies of the rat parotid gland also indicate that the majority of transglutaminase activity is associated with insoluble matrix elements.

These studies also indicate the presence of multiple forms of transglutaminase. In the lung, the matrix-associated enzyme (type B) differs from the cytosolic "tissue transglutaminase" (type C) found in many cells. In the parotid, three distinct chromatographic peaks of activity occur, although they have not yet been characterized.

Studies of fibrin clot formation catalyzed by factor XIIIa have shown that albumin at physiological concentrations has an important effect on the number of crosslinks and the stabilization of fibrin. Albumin induces an increase in γ-γ-dimer formation and an acceleration of amine incorporation. These effects appear to be at least partially dependent upon the reductive activity of the single sulfhydryl group present in albumin. Oxidative agents and metabolites, such as those present in tobacco smoke or generated during the inflammatory process cause a rapid loss of factor XIIIa activity. These findings suggest that the formation of a "temporary tissue matrix" during tissue repair is regulated by the balance of several opposing physiological factors.

PUBLICATIONS

Cooper H.L., Park, M.H. and Folk, J.E.: Hypusine formation; posttranslational modification in translation initiation factor eIF-4D. Methods in Enzymology 106:344-351, 1984.

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Gorman, J.J. and Folk, J.E.: Structural features of glutamine substrates for transglutaminases: Role of extended interactions in the specificity of human plasma factor XIIIa and the guinea pig liver enzyme. J. Biol. Chem. 259:9007-9010, 1985.

Hand, A.R. and Weiss, R.E.: Effects of streptozotocin-induced diabetes on the rat parotid gland. Lab. Invest. 51:429-440, 1984.

Hubbard, V.S., Wolf, R.O., Lester, L.A. and Egge, A.C.: Diagnostic and therapeutic applications of the bentiromide screening test for exocrine pancreatic insufficiency in patients with cystic fibrosis: A comparison with other tests of exocrine pancreatic disease. Dig. Dis. Sci. 29:881-889, 1984.

Lewis, M.S., Carmassi, F. and Chung, S.I.: Cooperative association of plasminogen with fibrinogen. Biochemistry 23:3874-3879, 1984.

Mazariegos, M.R. and Hand, A.R.: Regulation of tight junctional permeability in the rat parotid gland by autonomic agonists. J. Dent. Res. 63:1102-1107, 1984.

Mednieks, M.I. and Hand, A.R.: Cyclic AMP binding proteins in saliva. Experientia 40:945-947, 1984.

Park, M.H., Liberato, D.J., Yergey, A.L. and Folk, J.E.: The biosynthesis of hypusine: Alignment of the butylamine segment and source of the secondary amino nitrogen. J. Biol. Chem. 259:12123-12127, 1984.

Robbins, A.R., Oliver, C., Bateman, J.L., Krag, S.S., Galloway, C.J. and Mellman, I.: A single mutation in Chinese hamster ovary cells impairs both Golgi and endosomal functions. J. Cell Biol. 99:1296-1308, 1984.

Sariban, E., Oliver, C., Corash, L., Cossman, J., Jaffe, E., Gralnick, H.R. and Poplack, D.C.: Acute megakaryoblastic leukemia in childhood. Cancer 54:1423-1428, 1984.

Sellitti, D.F., Oliver, C. and Latham, K.R.: Antibodies to nuclear thyroid hormone-binding proteins. Antibody characterization and immunofluorescent localization. Exp. Cell Res. 158:223-236, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00001-33 LOBP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transglutaminases: Specificities, Functions and Biological Roles of Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Folk, J.E.	Chief, Enzyme Chemistry Section	LOBP NIDR
OTHERS:	Thacher, S.	Staff Fellow	LOBP NIDR
	Fesus, L.	Guest Researcher	LOBP NIDR

COOPERATING UNITS *(if any)*
Dr. Jeffrey J. Gorman, CSIRO, The Australian National Animal Health Laboratory, Geelong, Australia; Dr. Mary Lynn Fink, Baylor University, Waco, Texas

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Enzyme Chemistry Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.55	2.30	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the specificity and catalytic mechanism of transglutaminases are underway. The cellular and extracellular functions of the products of transglutaminase action are under investigation. Knowledge has been obtained as to the catabolic fate of these transglutaminase products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00028-18 LOBP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructure and Cytochemistry of Secretory Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hand, A.R.	Chief, LOBP	LOBP NIDR
OTHERS:	Oliver, C.	Research Biologist	LOBP NIDR
	Mednieks, M.I.	Senior Staff Fellow	LOBP NIDR
	Hani, S.	Guest Researcher	LOBP NIDR
	Baum, B.J.	Chief, CIPCB	CIPC NIDR
	Kousvelari, E.	Expert	CIPC NIDR
	Lustmann, J.	Guest Researcher	LOBP NIDR
	Scott, J.	Visiting Associate	CIPC NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.69	1.12	2.57

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic mechanisms of the secretory process are studied in cells of the rat pancreas, salivary and lacrimal glands. Techniques utilized include light and electron microscopy, enzyme- and immunocytochemistry, radioautography, and biochemistry. Major areas of investigation are: (1) the structure and function of the Golgi apparatus and GERL; (2) experimental pathology and lysosome function in salivary glands; (3) structure and permeability properties of junctional complexes in the rat parotid gland; (4) the role of salivary duct cells in protein reabsorption; (5) structure and cytochemistry of the salivary glands of the rat soft palate; and (6) parotid β-adrenergic receptor localization using labeled antagonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00049-14

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Function of Transglutaminases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chung, S.I.	Research Chemist	LOBP NIDR
OTHERS:	Cocuzzi, E.T.	Visiting Fellow	LOBP NIDR
	Kim, H.	Guest Researcher	LOBP NIDR
	Lee, K.N.	Guest Researcher	LOBP NIDR
	Carmassi, F.	Visiting Associate	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. D. Galanakis, SUNY, Stony Brook, NY; Dr. M. Lewis, DRS, NIH; Dr. A. Janus, SUNY, Stony Brook, NY

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.70	3.50	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The physiological function and mode of regulation of transglutaminases are being studied as to their role in the formation of a "temporary tissue matrix" (fibrin or fibrin-connective tissue) during tissue or bone fracture repair and in the modulation of specific cellular processes.

The physiological significance and biochemical mechanisms of factor XIIIa- and tissue- transglutaminase catalyzed cross-linking of plasmin inhibitor (α_2-PI) to fibrin and other matrix proteins are under investigation. The cross-linking of α_2-PI to fibrin plays a major role in stabilization of the "temporary tissue matrix" which is vital for the initial phase of cell migration and proliferation at wound sites. A number of factors in the plasma and tissue fluid can affect the fibrin stabilization, i.e., oxygen metabolites, sulfhydryls, and neutral proteases. We find that albumin plays an important role in the modulation of both fibrin-gel formation and catalytic activity of factor XIIIa as a co-factor and also provides -SH groups that are required. Oxygen metabolites derived from either external sources or from inflammatory cell exudates are found to inhibit both factor XIIIa and cellular transglutaminases. Sulfhydryl groups (-SH), on the other hand, induce activation of tissue matrix-associated transglutaminase in the lung. The cross-linked fibrin clot was found to be stable toward leucocyte proteases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00199-09 LOBP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Oliver, C.	Research Biologist	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR
	Dromy, R.	Visiting Fellow	LOBP NIDR
	Kleinman, H.	Research Chemist	LDBA NIDR
	Siraganian, R.	Chief, CI, LMI	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. A. Robbins, GB, NIADDK

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.29	2.00	2.29

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Secretory and endocytic processes in several cell types are currently under investigation. Cell dissociation and short term culture (up to 1 month) methods have been established for rat exorbital lacrimal, parotid and pancreatic acinar cells. These cultures are being used to study various aspects of the secretory process. Emphasis is placed on morphological, cytochemical and biochemical characterization of the cultured cells. Uptake and fate of both soluble phase and membrane bound markers by exocrine acinar cells are being examined in vivo and in vitro. The lysosomal system and its role in endocytic processes is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00217-07- LOBP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Salivary Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Wolf, R.O.	Dental Director	LOBP NIDR

COOPERATING UNITS *(if any)*
Hubbard, V.S., Physician, PM, NIADDK; Merril, C., Physician, BPB NIMH: Harrington, M.G., Physician, BPB, NIMH

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with mechanisms of production and control of extrinsic (i.e., saliva) and intrinsic (e.g., serum salivary isoamylase) salivary gland products. Human and animal saliva chemical constituents and mechanisms are evaluated in relation to health, disease and physiological state. Parotid salivary flow rate, protein content and enzymes are evaluated in normal subjects and in selected disorders. The intrinsic secretion of salivary isoamylase in serum of patients with cystic fibrosis of the pancreas has been studied. Diagnostic applications are being sought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00285-05 LOBP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Protein Secretion in the Rat Parotid

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mednieks, M.I.	Senior Staff Fellow	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR
	Siraganian, R.	Chief, CI	LMI NIDR
	Cheng, L.F.	Visiting Fellow	LOBP NIDR
	Chung, S.I.	Research Chemist	LOBP NIDR
	Cocuzzi, E.T.	Visiting Fellow	LOBP NIDR

COOPERATING UNITS *(if any)* Prof. R.A. Jungmann, Northwestern University Medical School; Dr. D.E. Philpott, NASA Ames Research Center; Dr. A.S. Fine, New York V.A. Medical Center; Dr. S. Wientroub, Tel Aviv Medical School; Dr. J.D. Castle, Yale University.

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.33	2.15	1.18

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Regulatory mechanisms of protein synthesis and secretion are investigated by combining biochemical and molecular biology techniques with morphological studies. New in vitro translation products whose synthesis is directed by mRNA after stimulation by agents whose action is mediated via the cyclase system, are identified using characterized monoclonal antibodies to acinar cell secretory proteins. Molecular hybridization studies are carried out using a cDNA probe prepared by the reverse transcription of poly A^+ RNA. Intracellular staging of stimulus-responsive secretory protein synthesis and processing is followed electron microscopically using immunological probes labeled with colloidal gold.

Cyclic AMP-dependent protein kinase (ATP: protein phosphotransferase, E.C. 2.7.1.37) activity, localization and endogenous substrate responses are being measured in various tissues, under different stimulation conditions ranging from in vitro effects of pharmacologic agents to changes resulting from altered gravity conditions of animals subjected to space flight. Comparisons of posttranslational modifying enzyme effects are made after measuring activities of transglutaminase. Exocytosis can be regulated by agents which either undergo receptor interactions or involve cell membrane adenylate cyclase directly. Mechanisms of action of these stimuli appear to be cyclic AMP-dependent reactions involving gene regulation by cyclic AMP-mediated phosphorylation of cellular proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00311-05 LOBP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Unusual Amino Acid, Hypusine: Mechanism of Formation and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Folk, J.E.	Chief, Enzyme Chemistry Section	LOBP NIDR
OTHER:	Park, M.H.	Staff Fellow	LOBP NIDR
	Abbruzzese, A.	Visiting Fellow	LOBP NIDR
	Neece, S.H.	Chemist	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. H.L. Cooper, NCI; Drs. D.J. Liberato and A.L. Yergey, NICHHD, NPMB

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Enzyme Chemistry Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.77	3.16	0.61

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The amino acid hypusine has been identified as a component of a single protein in all mammalian cells examined. This protein is eukaryotic translation initiation factor 4D (eIF-4D). Hypusine is formed posttranslationally from lysine and the butylamine portion of the polyamine, spermidine, followed by hydroxylation. These data demonstrate a novel polyamine metabolic pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00376-02 LOBP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Secretion, Purification and Characterization of Enzymes from Lingual Serous Glands.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Field, R.B.	Staff Fellow	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. Robert A. Boykins, FDA, Division of Biochemistry and Biophysics, Bethesda, Maryland.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.10	1.15	0.95

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to investigate the secretory process in von Ebner's gland of the rat tongue, we are studying the effects of cholinergic and α- and β- adrenergic agonists and antagonists and the adenylate cyclase activator, forskolin, on the secretion of lingual lipase and amylase. The amount of secretion is determined by in vitro incubation of the dissected glands with the agonists. The tissue and medium are assayed for lipase and amylase activity and portions of the tissue are taken for light and electron microscopy. Stimulation of secretion by these agonists was also studied in vivo by morphological examination and assay of enzyme activity of the gland at various times after injection.

Lingual lipase and amylase were purified from von Ebner's gland and analyzed for amino acid and carbohydrate content. The isoelectric point of amylase was determined. Purified enzymes will be used to prepare antibodies and to further characterize lingual lipase and amylase.

ANNUAL REPORT OF THE LABORATORY OF ORAL MEDICINE,

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Laboratory of Oral Medicine studies the etiology and pathogenesis of both systemic diseases and diseases of the soft tissue of the oral cavity. Emphasis is on: (1) viral infections such as herpes simplex virus; (2) endocrine diseases, especially virus-induced diabetes mellitus; (3) autoimmune disorders; and (4) ulcerative and malignant lesions of the oral cavity. The program is disease oriented and highly interdisciplinary. The Laboratory is made up of investigators who are trained in a variety of disciplines including virology, immunology, pathology, molecular biology, and clinical medicine and dentistry.

Studies have continued on the projects discussed in last year's Annual Reports. Work on a recombinant DNA vaccine against herpes simplex virus (HSV) has continued. In mice the vaccine prevents the majority of the animals from developing a latent infection when challenged with live HSV. Long-term protection studies are now underway. In addition, a model for studying the reactivation of HSV in humans has been developed and will be investigated in-depth over the next year. In particular studies will be initiated on preventing reactivation in this model by use of antiviral drugs. In the area of virus-induced diabetes two new model systems have been developed. Coxsackie B viruses were shown to produce a transient type of diabetes in non-human primates and a slow virus (scrapies), which causes central nervous system disease, was shown to produce glucose abnormalities in hamsters. In the area of autoimmunity good progress has also been made. Studies from the laboratory have shown that certain autoantibodies (monoclonals) made against viruses can recognize antigens in perfectly normal uninfected tissues. In terms of mechanisms, this work suggests that cross-reactivity or molecular mimicry might be one of the factors responsible for triggering autoimmune disorders. In addition, a major effort is being put into studies on the role of Epstein-Barr virus, another member of the herpes family, in triggering the production of autoantibodies.

Since last year several key members of the laboratory have left: Dr. Ji-Won Yoon, who worked for many years on virus-induced diabetes; Dr. Kenneth Cremer, who headed up our studies on a recombinant DNA vaccine against herpes simplex virus; and Dr. Patrick McClintock, who led our studies on isolating autoantigens with monoclonal autoantibodies. A number of new people have been recruited to take over and expand upon the ongoing projects. More emphasis will be placed on molecular biology and some new projects, including the development of transgenic mice, will be initiated.

Since last year, a number of new techniques were introduced into the laboratory and existing ones modified. Specific techniques include: (1) antibody screening of high-efficiency cloning of cDNA in the expression vector lambda gt11; (2) high pressure liquid chromatography (HPLC), using gel filtration, ion exchange, and reverse phase; (3) immunoblotting on nitrocellulose for detection of autoantigens in cell and tissue extracts and HPLC eluates; (4) modification of the 3T3 cell transformation assay to include co-transfection into NIH/3T3 cells of genomic DNA with a selectable plasmid and injection of drug resistant transformants into nude mice to increase the efficiency of detection of a wider range of oncogenes; (5) filter immunoassay for the in situ detection of insulin secreting beta cell colonies; (6) transient expression assay to detect beta-galactosidase in cells co-transfected with beta-gal and activated oncogene containing plasmids; (7) biotinylation of various molecules including antibody, thyroglobulin, tetanus toxoid, insulin and cythochrome C; (8) fluorescein-activated cell sorting (FACS); (9) separation and characterization of lymphocyte subsets by monoclonal reagents and FACS; (10) EBV nuclear antigen detection by indirect complement binding assay; (11) transformation of lymphocytes by a coupled calcium phosphate-polyethylene glycol method; (12) probing of genomic DNA for foreign DNA using species specific repetitive DNA probes; (13) use of highly purified antigens to monitor antigen-specific proliferation and preferentially propagate antigen specific cells; and (14) preparation and analysis of anti-idiotypic antibodies and anti-anti-idiotypic antibodies.

The laboratory is involved in a number of collaborative projects. Past collaborations continue and a number of new ones have been initiated. Active projects include: (1) studies on the state of the viral genome during latency (Wistar Institute); (2) experimental reactivation of HSV in humans with UV light (Laboratory of Clinical Investigation, NIAID); (3) immunochemistry of monoclonal antibodies (Loma Linda University School of Medicine); (4) evaluation of cross-reactivity between viruses and normal cells (Scripps Clinic and Research Foundation, Walter Reed Army Institute of Research, and the Wistar Institute); (5) lymphocytic choriomeningitis virus induced thyroiditis (Scripps Clinic and Research Foundation); (6) slow viruses and diabetes (NINCDS, NIH); (7) the binding of human monoclonal autoantibodies to glycolipids and determination of the structure recognized by these antibodies (Laboratory of Biochemical Pharmacology, NIADDK); (8) FACS analysis of monoclonal antibodies (Department of Infectious Diseases, Walter Reed Army Medical Center); (9) immunological abnormalities in diabetes (Mt. Sinai Medical Center, New York City); (10) flow cytometry analysis of lymphocytes and sorting (NIADDK); (11) characterization of antibodies to human thyroglobulin and microsomes (Division of Endocrinology, Mt. Sinai School of Medicine, New York); (12) polymyositis and Jo-1 antigen (Arthritis Branch, NIADDK); (13) testing of DNA from transformed lymphocytes for evidence of its oncogenic potential (Department of Veterinary Microbiology, Washington State University); (14) human lymphoblastoid cell lines secreting monoclonal autoantibodies (Institute of Microbiology, University of Pisa, Pisa, Italy); (15) characterization of antigenic variants of Coxsackievirus B_4 (University of the Commonwealth of Virginia); (16) generation of monoclonal antibodies against osteonectin and osteogenin (Mineralized Tissue Research Branch, NIDR); (17) development of an antigen capture assay for detecting IgM anti-Coxsackievirus B_4 antibodies in human sera (St. Thomas' Hospital, London); (18) characterization of a heart antigen with which one of the anti-Coxsackievirus B_4 monoclonal antibodies reacts (Johns Hopkins University); (19) testing the efficacy of vaccinia virus recombinants expressing HSV glycoprotein D as a vaccine against HSV infection (Laboratory of Viral Diseases, NIAID); (20) construction and testing of recombinant vaccine vectors that express the glycoprotein B genes of HSV-1 (City of Hope Medical Center, Duarte, California); and (21) long-term prospective study on newly diagnosed insulin-dependent diabetes (Mt. Sinai Medical Center, New York City).

Some of our more important findings since last year's Annual Report are summarized below:

I. HERPES SIMPLEX VIRUS AND ONCOLOGY

1. During the last year we continued our studies on the efficacy in mice of a new vaccinia virus recombinant vaccine expressing herpes simplex virus (HSV) type 1 glycoprotein D. In humans, herpes simplex virus causes a primary infection and then often a latent ganglionic infection that persists for life. Because these latent infections can recur periodically, vaccines are needed that can protect against both primary and latent HSV infections. Infectious vaccinia virus recombinants that contain the HSV type 1 glycoprotein D gene under control of defined early or late vaccinia virus promoters were constructed by colleagues at NIAID. Tissue culture cells infected with these recombinant viruses synthesized a glycosylated protein that had the same mass (60,000 daltons) as the glycoprotein D produced by HSV-1. Immunization of mice with one of these recombinant viruses by intradermal, subcutaneous, or intraperitoneal routes resulted in the production of antibodies that neutralized HSV-1 and protected the mice against subsequent lethal challenge with HSV-1 or HSV-2. Immunization with the recombinant virus also protected the majority of the mice against the development of a latent HSV-1 infection of the trigeminal ganglia. This is the first demonstration that a genetically engineered vaccine can prevent the development of latency. The duration of immunity against HSV produced by the recombinant virus is now under study.

2. A significant problem, which has delayed progress in the understanding and treatment of HSV infection, has been the lack of a manipulable human model. Over the past year we have experimentally explored the reactivation of latent HSV in humans by UV light. Five otherwise healthy patients between 18 and 50 years of age, with culture confirmed HSV infection involving the buttocks and sacral area, were studied. We found that treatment with UV light triggered cutaneous HSV infection in over 60% of the attempts. There was considerable variation among individuals, but in some individuals reactivation was successful on every attempt. These findings point to a new approach that might be helpful to patients suffering from a latent infection with frequent recurrences; that is, the identification of factors that cause reactivation. Once specific factors are identified, it might be possible to avoid them or devise methods of intervention. For example, in the case of UV light there might be a "window of vulnerability" during which time treatment with a short course of an antiviral agent such as acyclovir might prevent the development of a recurrence. If this is the case, then treatment could be directed at high risk individuals. If successful, this would reduce the frequency of recurrences while avoiding the use of chronic suppressive antiviral treatment.

3. During the past year we have explored one of the possible biological implications of the relationship between HSV, DNA and the c-Ha-ras1 oncogene. To briefly summarize, last year we reported that discrete stretches of 40-60 nucleotides within the immediate-early (IE) transcriptional enhancer of HSV were homologous to an essential regulatory region of the oncogene. Deletion of this oncogene region abolished its capacity to transform NIH/3T3 cells; however, its replacement by the HSV homologous stretch restored the transforming potential of the oncogene. These results led us to speculate that during a naturally occurring infection, illegitimate recombination events could occur between HSV DNA and the normal homologue of c-Ha-ras1, and that possibly, these recombination events could underlie the hit-and-run nature of HSV-induced transformation. We tested this hypothesis in two ways. First, we

measured the ability of plasmids carrying the HSV IE enhancer sequences to transform NIH/3T3 cells. The rationale being that, if the enhancer integrated by recombination into the regulatory region of *c-Ha-ras1*, it could induce expression of the protooncogene, and lead to the transformed phenotype. Second, we cloned the normal homologue of *c-Ha-ras1* into a lambda vector that carried two *su* mutations, and grew this clone in *E. coli* cells containing the HSV IE enhancer cloned into the plasmid piVX, that carries a synthetic supF gene. Any phage progeny capable of growth in *su*° cells should arise either by reversion of the double *su* mutation or by recombination at quasi-homologous sites between phage and plasmid DNA and formation of a pseudo-su^+ bacteriophage. The recombinants can easily be distinguished, since the reversion frequency is of the range of 10^{-12}. Both of these approaches gave the same experimental answers, i.e., that the HSV IE enhancer and the *c-Ha-ras1* oncogene did not recombine with a measurable frequency.

4. In the area of oral tumors, investigators in the laboratory found that T cells from patients with oral tumors exhibited a decreased response to T cell mitogens. Of particular interest was the observation that this defect was found in the very early stage of the disease. Investigation of lymphocyte subsets suggest that NK cell activity may be increased in these patients.

II. VIRUS-INDUCED DIABETES MELLITUS

1. Insulin-dependent diabetes mellitus (IDDM) is due to destruction of insulin-producing beta cells in the pancreas. Both environmental insults (e.g., viruses and chemicals) and autoimmunity have been implicated as possible causes. In mice, encephalomyocarditis (EMC) virus and members of the Coxsackievirus B group can infect beta cells and cause diabetes. In humans, several lines of evidence suggest that Coxsackieviruses can cause or at least be the final insult in triggering occasional cases of IDDM. The number of well documented cases, however, is small and a non-human primate model would be of value. Several years ago an investigation was initiated to study the susceptibility of different species of monkeys to EMC virus- and Coxsackievirus-induced diabetes mellitus. Cynomolgus, Rhesus, and Cebus monkeys failed to show glucose tolerance or insulin secretion abnormalities after infection with EMC virus or Coxsackievirus B_4. Patas monkeys also showed no abnormalities after infection with EMC virus. However, Patas monkeys infected with Coxsackievirus B_4 or treated first with a subdiabetogenic dose of streptozotocin and then infected sequentially with Coxsackieviruses B_4 and B_3 showed transient elevation of glucose tolerance tests, depressed insulin secretion and glucose in the urine. These experiments in non-human primates add support to the earlier studies in mice and humans that under certain circumstances Coxsackieviruses can cause abnormalities in glucose homeostasis.

2. Neural regulation of the pancreatic islets contributes to plasma glucose homeostasis. Over the last year we initiated studies in collaboration with Dr. Carleton Gajdusek's group to determine whether viruses that infect the central nervous system (CNS) can cause diabetes. Scrapies is a slow virus of the CNS. Our studies showed that hamsters infected with scrapies develop diabetes as evaluated by abnormalities in glucose tolerance tests. Radioimmunoassays revealed a decrease in plasma immunoreactive insulin. The mechanism for these changes is now under investigation.

3. A possible contribution of the immune system to the pathogenesis of virus-induced diabetes mellitus was investigated using the D-variant of encephalomyocarditis (EMC-D) virus. Studies on the F_1 and backcross progeny of susceptible and resistant strains of mice gave no sugestion of a linkage between susceptibility and the major histocompatibility locus. Immunosuppression by anti-lymphocyte serum did not prevent the induction of EMC-induced diabetes. Athymic nude mice infected with EMC virus showed a nearly identical diabetogenic response as compared to heterozygous littermates. Passive transfer of lymphocytes from mice made diabetic with EMC virus into normal mice failed to produce diabetes. From these and other studies, we conclude that the development of EMC-induced diabetes is due to the direct destruction of beta cells by the virus and that the contribution of the immune response to the pathogenesis of this disease is at the most, minor.

4. The NIH/3T3 cell transfection system is being is used to detect the presence of transforming oncogenes in human insulinoma tumors and the RINm5F rat insulinoma. The ultimate goal is to determine whether a single activated oncogene is found in all tumors of beta cell origin. Genomic DNA extracted from a human insulinoma and from the RINm5F cell line produced transformation frequencies in the range of 0.03 to 0.05 foci/ug DNA. Transformed NIH/3T3 cells are being analyzed by restriction enzyme digestion and Southern blot hybridization to determine if the transformants have acquired rat or human DNA sequences and whether these sequences are related to known oncogenes.

5. Previously we prepared a panel of monoclonal antibodies that neutralized Coxsackievirus B_4. Using these antibodies, considerable antigenic diversity among the natural isolates of Coxsackievirus B_4 was observed. Plaque-purified preparations of Coxsackievirus B_4, when grown in the presence of neutralizing monoclonal antibodies, yielded antigenic variants at a frequency of 10^{-4} to 10^{-5}. Over the last year, we initiated experiments to evaluate the capacity of different assays to detect and quantitate spontaneous antigenic variants in the apparent absence of selective pressure and to study the conservation of epitopes on clinical isolates of Coxsackievirus B_4. Analysis of 47 clinical isolates with 16 monoclonal neutralizing antibodies revealed highly conserved, moderately conserved and poorly conserved epitopes. Upon passage of Coxsackievirus B_4 in tissue culture, certain epitopes disappeared and others appeared in the apparent absence of selective pressure. Changes in epitopes often exceeded a frequency of 10^{-2}. Despite considerable variation in individual epitopes, we conclude that the overall antigenic composition of Coxsackievirus B_4, as measured by the capacity of hyperimmune serum to neutralize clinical isolates, remains fairly constant.

6. Paratopes of some antiviral antibodies might be mirror images of the viral antigen to which they bind. Therefore, an "internal image" anti-idiotypic antibody against an antiviral antibody may exhibit some of the same properties as the viral antigen. Such antibodies might be used as probes of virus receptors and as vaccines against viral infections. We made anti-idiotypic antibodies in rabbits against three monoclonal neutralizing antibodies with specificities for independent epitopes on Coxsackievirus B_4. Each of the anti-idiotypic antibodies was shown to be specific for the immunizing antibody and to inhibit the function of that antibody. These anti-idiotypic antibodies were then tested for their ability to recognize receptors for Coxsackievirus B_4. None of the anti-idiotypic antibodies bound to the surface of Coxsackievirus B_4 receptor-positive cells, nor did they inhibit the

attachment of radiolabeled Coxsackievirus B_4 to cells. The anti-idiotypic antibodies did induce an anti-anti-idiotypic antibody response in mice when measured by a competitive inhibition assay; but, at best, only minimal virus neutralizing activity was found. Our results suggest that not all monoclonal neutralizing antibodies interact with cellular attachment sites on viruses and that not all anti-idiotypic antibodies are capable of eliciting a strong antiviral response.

III. AUTOIMMUNITY AND VIRUSES

1. Molecular mimicry may be one of the mechanisms by which viruses can trigger an autoimmune response. The idea is that antibodies raised against certain viral antigens may react with normal host cell antigens. To study this possibility, over 600 monoclonal antiviral antibodies made against 11 different viruses were screened against a panel containing up to 14 different uninfected normal mouse organs. Twenty-one of these antiviral antibodies, or approximately 3.5%, reacted with cells in specific organs. Several of these antibodies were of the multiple-organ reactive type and recognized antigens in more than one organ. It is concluded that the reactivity of monoclonal antiviral antibodies with normal tissues is a common phenomenon. Purification and characterization of some of these autoantigens is now in progress.

2. Over the past few months we developed a method for isolating human B cells that make antibodies to self antigens. We succeeded in isolating antigen- specific B cells by exploiting their binding to biotinylated molecules and by sorting with a fluorescein-activated cell sorter (FACS). We showed that the B lymphocytes selected by FACS can be easily transformed by Epstein-Barr virus (EBV) and that the immortalized cells produce the type of antibody for which they have been selected. This method provides a potentially powerful tool for the isolation and production of specific human monoclonal antibodies.

3. Using EBV-mediated transformation of human peripheral blood B lymphocytes derived from patients with recently diagnosed IDDM, lymphoblastoid cell lines secreting autoantibodies reactive with human T lymphocytes have been established. One of these designated DCT-1 has been extensively studied. The autoantibody produced by this line is an IgM (k) and reacts with the surface of T cells. By FACS analysis, DCT-1 has been found to react with 75% of circulating T helper cells and 50% of circulating T cytotoxic/suppressor cells. DCT-1 does not recognize surface Ig, HLA-A,B,C, or HLA-DR antigens. This implies that it recognizes an antigen common to the T helper and T cytotoxic/suppressor lineages. Studies are now underway to determine if the DCT-1 positive subset of T cells subserves an identifiable function within the immune system or, alternatively, whether there is any linkage between the presence of DCT-1 positive T cells and autoimmune diseases.

4. Peripheral blood lymphocytes from autoimmune patients and controls have been maintained in culture in order to examine the prevalence and characteristics of spontaneously proliferating B lymphocytes. The autoimmune diseases under study include IDDM, polymyositis, dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis and Sjogren's syndrome. Of the 38 individuals examined, cells from 19 showed proliferation from 4 to 8 weeks after the cultures were initiated. The frequency of transformed cells was as

high as 1 in 10^6 mixed lymphocytes or 2 in 10^5 B lymphocytes. EBV appears to be the transforming factor in at least some of these cell lines. The role that EBV plays in the etiology and pathogenesis of these autoimmune diseases is now under investigation.

5. Antibodies that react with pancreatic islets are frequently found in the serum of patients with newly diagnosed IDDM. What is perhaps not as fully appreciated is that autoantibodies that react with a variety of other organs (e.g., gastric mucosa, adrenals, anterior pituitary, lymphocytes) are also sometimes found in the serum of IDDM patients. Autoantibodies that react with the thyroid are particularly common. The nature of this multiple-organ autoreactivity of serum has never been fully explained. There are at least two possibilities. One is that the serum of IDDM patients contains a number of organ-specific antibodies. The second is that the serum contains antibodies that recognize common proteins or epitopes in different organs. Using hybridoma technology or EBV-induced transformation, it is now becoming possible to address this question by studying the reactivity of human monoclonal autoantibodies. Over the last year, we developed a rapid screening procedure for detecting human monoclonal autoantibodies that react with pancreatic islets, and showed that many of these autoantibodies also react with the thyroid. Based on these and other studies we conclude that lymphocytes capable of making autoantibodies that react with both the pancreas and thyroid are common in the human B-cell repertoire.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE00080-12

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diabetes Mellitus and Other Viral Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*
Laboratory of CNS Studies, NINCDS, NIH

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 9.02	PROFESSIONAL: 4.38	OTHER 4.64

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with virus-induced diabetes and virus-induced autoimmunity in animals and man. In the area of virus-induced diabetes, two new models have been developed. Coxsackievirus B4 has been shown to produce a transient type of diabetes in nonhuman primates characterized by abnormalities in glucose tolerance and insulin secretion. Patas monkeys were more susceptible than other strains. In hamsters, a slow virus infection (i.e., scrapie), which causes central nervous system disease, produced marked abnormalities in glucose tolerance, but no apparent damage to the pancreas. The glucose abnormalities appear to be related to virus-induced central nervous system damage. Studies have continued on diabetes induced in mice by encephalomyocarditis (EMC) virus. The role that immunopathology plays in the development of this disease was examined and, based on a number of studies, it was concluded that the diabetes is due to direct destruction of pancreatic beta cells by EMC virus and that the contribution of the immune response to the pathogenesis of this particular disease is at the most minor. In the area of virus-induced autoimmunity, the role that viruses may play in triggering autoimmune disease was evaluated by studying over 600 monoclonal antiviral antibodies. These antibodies were screened for reactivity against normal uninfected tissue. It was found that approximately 3.5% of the antiviral antibodies reacted with perfectly normal tissue. It is concluded that this type of cross-reactivity or "molecular mimicry" is a common phenomenon. In addition, a major effort is being put into studies on the role of Epstein-Barr virus in triggering the production of autoantibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00123-12

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Herpes Simplex Virus and Other Oral Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

A. Puga	Expert	LOM,NIDR
K.T. Cremer	Sr. Staff Fellow	LOM,NIDR
P.R. Brayton	Staff Fellow	LOM,NIDR
D. Eskinazi	Expert	LOM,NIDR
J. Gomez	Visiting Fellow	LOM,NIDR
J. Perna	Med. Staff Fellow	LOM,NIDR
A.L. Notkins	Medical Director	LOM,NIDR

COOPERATING UNITS *(if any)*
Laboratory of Viral Diseases, NIAID
Laboratory of Clinical Investigation, NIAID

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 8.02	PROFESSIONAL: 4.13	OTHER: 3.89

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A recombinant vaccinia virus which expresses glycoprotein D of HSV-1 has been tested for its efficacy as a vaccine to prevent primary and latent infections with HSV-1 and HSV-2. Mice vaccinated with the recombinant virus were challenged with a lethal dose of HSV-1 or HSV-2. Over 90% of the mice were protected in both cases. To determine whether vaccination would prevent the development of a latent HSV infection, mice were vaccinated intradermally (tail route) with vaccinia-HSV-1 gD recombinant and four weeks later challenged with HSV-1 (KOS) by the lip route. Approximately two-thirds of the ganglia from vaccinated mice were protected from the development of a latent infection, in contrast to less than 5% protection in the control mice. This is the first demonstration of the effectiveness of a genetically engineered vaccine to prevent the establishment of a latent HSV infection.

Using in situ hybridization techniques, the presence of HSV gene transcripts could be demonstrated in trigeminal ganglia of latently infected mice. To identify the gene(s) expressed, polyadenylated mRNA from trigeminal ganglia of latently infected mice was used as a template to synthesize labeled cDNA molecules which could be used as probes for Southern blots of cloned HSV DNA restriction fragments. mRNA samples were obtained from mice of ages ranging up to 24-month old animals and mRNA from trigeminal ganglia of uninfected mice was used as a control. Probe sequences capable of hybridizing with some viral fragments but not with others were found in the samples from animals of all ages but not in the controls. The viral fragments detected were always the same, regardless of the age of the mice used, and finer mapping of the restriction fragments involved indicates that transcription of the viral genome during latency is restricted to two, possibly three of the HSV immediate early genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00309-05

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Autoimmunity and Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B. Prabhakar	Sr. Staff Fellow	LOM,NIDR	K.F. Salata	Staff Fellow	LOM,NIDR
A.L. Notkins	Medical Director	LOM,NIDR	Y. Uchigata	Visiting Fellow	LOM,NIDR
G.P. Allaway	Visiting Fellow	LOM,NIDR			
P. Casali	Visiting Scientist	LOM,NIDR			
D.W. Drell	Staff Fellow	LOM,NIDR			
K. Essani	Visiting Fellow	LOM,NIDR			
E.L. Oates	Staff Fellow	LOM,NIDR			
J.B. Shiroky	Visiting Associate	LOM,NIDR			

COOPERATING UNITS *(if any)*

Mt. Sinai School of Medicine, NIDR, NY

Arthritis and Rheumatism Br., NIADDK, NIH

LAB/BRANCH Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 13.76	PROFESSIONAL: 5.52	OTHER 8.24

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have immortalized human B lymphocytes using hybridoma techniques and Epstein-Barr virus transformation. These cells secrete autoantibodies that react with multiple organs. Some of the autoantibodies have been used in affinity columns to isolate and characterize the autoantigens with which they react. Monoclonal anti-idiotypic antibodies against some of the autoantibodies have been made. These reagents have been used to screen sera from patients with various autoimmune disorders to see whether there is any difference in the levels of autoantibody associated idiotopes. Some of the idiotopes are generally found in all sera and some others appear to be associated with specific autoimmune disorders. Recently we have made anti-anti-idiotypic antibodies. These antibodies are being used to further understand the nature of autoantigens and autoimmune-response, and to study the autoreactive B cell repertoire in humans.

MINERALIZED TISSUE RESEARCH BRANCH SUMMARY REPORT

The 1984-1985 year marked the second year of operation for the Mineralized Tissue Research Branch. This is the only NIH laboratory dedicated to basic and applied research on the bones and teeth. This year was marked by extensive laboratory renovations to the Branch facilities in Building 30 and by major research advances in: the molecular biology of bone and enamel; the cellular biochemistry of the osteoblast; the differential expression of noncollagenous proteins in mineralizing and non-mineralizing connective tissue; the structure and metabolism of the cartilage and granulosa cell proteoglycans; the isolation of osteogenin, the bovine bone inductive protein; model studies of matrix vesicle calcification; and connective tissue protein structure and function. These significant research achievements are detailed below.

Skeletal Matrix Biochemistry Section

It is now established that some of the noncollagenous proteins in the extracellular matrices of the bones and teeth are either unique gene products of these tissues or are highly enriched in them compared to other, non-mineralizing connective tissues of the body. Each of these special matrix proteins is a biosynthetic product of the cells that form the mineralized tissues and each, in some way, contributes to the unique biological properties of the bones and teeth. Further, these noncollagenous proteins are modulated in heritable and acquired disorders of the skeleton and are useful as markers of mineralized tissue pathology. Study of such phenomena is organized in the Section along three interdependent disciplinary lines of investigation; protein chemistry and immunochemistry, cell biology and molecular biology.

In the past year, several monoclonal lines against purified bovine osteonectin were generated, three of which have been successfully expanded and fully characterized. Each of the three antisera recognize distinctly different series of animal species osteonectin and are therefore thought to be directed against different epitopes within the osteonectin molecule. One monoclonal (ON-6) recognizes the osteonectin from all species tested and appears to be directed against a highly conserved region of the molecule. Interestingly, the monoclonal antibodies do not cross-react on Western blots with an osteonectin-like protein found in low abundance in the bones of one bovine model of osteogenesis imperfecta. This result implies that a family of osteonectin-like proteins may be produced by different tissues in the body, one of which, the bone osteonectin, may be specific to normal bone.

Two radioimmune assays (RIA) have been developed, one using antisera directed against human osteonectin, the other against bovine osteonectin (but which is fully cross-reactive with human). The results suggest that the apparent level of osteonectin in human serum is a function of the assay (antisera) used for quantitation. These results may well reflect the ability of one assay to detect more osteonectin-related products than another. The anti-bovine osteonectin-based RIA has been optimized for use in cell culture studies in our Section's bone cell biology group.

The small proteoglycans from bovine bone, tendon and cartilage were studied comparatively and the core proteins characterized in part. The results suggest that each tissue contains two distinctly different small proteoglycans with non cross-reactive (immunochemical critera) protein cores (PGI and PGII), but that each class (PGII, for example) of proteoglycan may vary in different tissues. For example, the bone and tendon PGII core proteins have similar antigenic determinants and crude peptide maps, but have completely different susceptibility patterns to V8 protease digestion. Further, the tendon PGII species in at least one form of bovine osteogenesis imperfecta appears more related to bone PGII in this regard than to the PGII core protein of tendon from normal calves. At present, we do not know if these subtle differences are due to the presence of different but evolutionarily related primary gene products or to tissue-specific post-translational modifications of a single PGII core protein.

Studies in bone cell biochemistry have shown that the cell culture system described last year, when used to obtain bone cells from a variety of species including calf, dog and man, routinely provides cultures that are characterized by high levels of alkaline phosphatase and a thick extracellular matrix that mineralizes in vitro. These bone cell cultures respond to a variety of hormonal and pharmacological factors such as parathyroid hormone, prostaglandins E_1 and E_2 Interleukin-1, forskolin, isoproteronal, and 1,25 dihydroxyvitamin D_3, but not to calcitonin. Receptors for 1,25 $(OH)_2D_3$ were identified using a cytosol assay and adult human cells were found to contain more 1,25 $(OH)_2D_3$ receptors than fetal human bone cells. Further, induction of the enzyme 25-hydroxyvitamin D_3-24-hydroxylase was proportionately higher in adult than in fetal bone cells. The lymphokine, IL-1, was mitogenic to human bone cells at doses from 1 to 10μ/ml and increased protein synthesis, particularly of fibronectin and the bone proteoglycans. These data indicate that the human bone cell culture system can be used to provide valuable insights into mechanisms operative in the metabolic control of bone formation. Further, preliminary studies conducted this year demonstrated that human bone cells can be grown in 5% human serum without apparent cytotoxic effect. Thus, it now appears feasible to apply cell culture techniques to study a variety of conditions exemplary of perturbances in human bone metabolism in vitro.

Biosynthesis experiments conducted this year have indicated that non-mineralizing connective tissue fibroblasts synthesize osteonectin and bone proteoglycan (PGII) cross-reactive proteins in vitro, even though these proteins are found only at very low levels in nonmineralized tissues in vivo. However, incorporation of these two proteins into the cell layer/extracellular matrix is reduced in fibroblast cultures compared to that of bone cell cultures. In addition, preliminary results indicate that the osteonectin cross-reactive protein produced by fibroblasts does not immunoprecipitate normally with a monoclonal antibody which fully immunoprecipitates bone cell osteonectin. Other data suggest that the osteonectin produced by some osteogenesis imperfecta bone cells may not immunoprecipitate normally compared to controls using this monoclonal antibody reagent. In view of these data and their potential significance, the chemical bases for these differences is currently under active investigation.

Cultures of bovine odontoblast-like cells were established this year by explanting small pieces of peripheral pulp (obtained from unerupted, growing teeth) onto plates pre-coated with basement membrane components. Cells with an epithelioid morphology predominated these cultures. These cells were alkaline phosphatase positive and formed on extracellular matrix that mineralized *in vitro*. The matrix contained type I (with no type III) collagen, osteonectin, bone proteoglycans and dentin phosphophoryn, all of which are found in normal dentin *in vivo*. Thus, the culture conditions utilized apparently selected pre-odontoblastic cells from the growing pulp and provided them an environment to express their potential for cytodifferentiation and dentinogenesis. This system should afford an excellent model to study regulation of dentin formation by specific physiological or pharmacological agents.

Major advances were made this year in the molecular biology of bone and tooth matrix proteins. cDNA libraries were prepared from polyA+RNA isolated from both bovine bone cell cultures and ameloblast-rich tissue and then inserted into the expression vector λgt11. The libraries were screened for the presence of recombinant product using polyclonal antisera directed against bovine osteonectin, small bone proteoglycan (PGII) and amelogenin. This resulted in each case in the subsequent isolation of several cDNA clones encoding these proteins. Because of the limited amino acid sequence data available for the bone matrix proteins, these clones were identified by hybrid-selected translation experiments followed by immunoprecipitation with specific antisera. The hybrid-selected preosteonectin molecule had an M_r of 47,000, while the clone for the bone proteoglycan hybridized to messages that translated two core proteins at M_r's of 38,000 and 40,000 on SDS gels. The cDNA for osteonectin is currently being sequenced and preliminary data indicate considerable peptide extension beyond the known amino terminus of the molecule. On Northern analysis, the osteonectin message was found to be 2.0 kb long, while the proteoglycan message was of two sizes, 1.4 and 1.8 kb. In general, the distribution of messenger RNA for these two bone matrix proteins was consistent with the presence of message in different connective tissues indicating a transcriptional control mechanism for the expression of these genes. Whether the two forms of core protein for the bone proteoglycan (PGII) are distributed differently in different connective tissues remains unknown at present.

In the case of the enamel matrix genes, cDNA specific for bovine amelogenin was also identified by DNA sequence analysis. In comparing the known amino acid sequence for one polypeptide isolate of the bovine amelogenin complex to the amino acid sequence deduced from the cDNA, a complete match was observed for residues 33-168 (residues 1-32 were missing from the DNA clone) and for residues 164-187. Interestingly, for this particular cDNA, a divergence of sequence (protein *vs*. cDNA) was detected in residues 139-163. Hybrid-selected translation experiments showed that the cDNA clone for this amelogenin selected at least 4 protein species ranging in size from 22,500 to 25,000 daltons. Preliminary Southern analysis with this clone suggested only one copy of the amelogenin gene per haploid genome. All the data taken together indicate that the polydispersity of newly synthesized amelogenin protein in developing enamel tissue arises from differential or alternatiave splicing of amelogenin RNA.

Finally, genomic DNA encoding osteonectin was isolated from bovine genomic libraries using the osteonectin cDNA as probes. In an initial screen of 500,000 recombinants, ten clones were identified and purified, two of which were amplified and studied in detail. These two clones had extensive overlap by restriction map analysis. When heteroduplexes of one of these were analyzed by electron microscopy, it was clear that the genomic clone contained 11.0 kb of the osteonectin gene and approximately 9.0 kb of 3' flanking region. The gene contains at least six introns, all quite large (between 0.8 and 2.0 kb) interspersed by seven exons that are small (~0.15 kb) and uniform in size. Future work will map further the properties of this gene and its expression in skeletal tissue.

Proteoglycan Chemistry Section

Proteoglycans are ubiquitous components of connective tissue, where they play major roles in determining the physical properties of these tissues, and where they serve a number of biological functions. Proteoglycans have complex structures and differ markedly in size and composition. The research efforts of the Proteoglycan Chemistry Section are directed toward elucidating the structure, biosynthesis, catabolism and functions of proteoglycans in various tissues and their alteration in pathological states.

The following studies were done this year with proteoglycans from the Swarm rat chondrosarcoma: (a) The tryptic peptide in the hyaluronic acid-binding region which contains the epitope for the 1-C-6 monoclonal antibody has been purified and sequenced through the first 20 residues, about 60% of the total. (b) The chymotryptic peptide in the hyaluronic acid-binding region which contains the epitope for the 5-C-4 monoclonal antibody has been identified and purified for further studies. (c) Both of these epitopes were shown to be sequestered in the native hyaluronic acid-binding region structure (the amino terminal portion of the proteoglycan) and are highly conserved throughout the evolutionary tree. (d) The 5-C-4 epitope was shown to be present in link protein as well as the hyaluronic acid-binding region of the proteoglycan core protein suggesting that it may reside in a region involved in the binding of these two molecules to hyaluronic acid. (e) The kinetics of incorporation of label from ^{3}H-glucose into different sugars in the chondroitin sulfate chain indicated that xylosylation of the core protein may occur 5-10 min earlier than when the rest of the chondroitin sulfate chain is added; thus xylosylation may be an early Golgi event.

The following studies were conducted with articular cartilage explant cultures: (a) The polypeptide catabolin, related to Interleukin 1, causes identical changes in proteoglycan metabolism as the bacterial lipopolysaccharide endotoxin, namely decreased proteoglycan synthesis, increased catabolism of proteoglycans from the matrix, increased hyaluronate synthesis after long exposure (10-20 days), and increased prostaglandin synthesis. (b) The induced changes by the endotoxin are reversible after intermediate times of exposure (5-15 days) but are only partially so after longer exposure times, suggesting that some chondrocytes in the tissue may have altered their phenotypic expressions.

Additional studies were done on proteoglycan metabolism in rat ovarian granulosa cells: (a) A new separation procedure using Triton X-100 gradients in 4 M guanidine HCl on octyl-Sepharose columns was developed which appears to separate proteoglycan species on the basis of differences in the hydrophobicity of their core proteins. (b) The intracellular degradation pathways for cell surface proteoglycans were further resolved. Leupeptin, an inhibitor with trypsin-like protease activities, slowed the fast pathway 1, but did not prevent final degradation of the proteoglycan species in functional lysosomal compartments. However, in pathway 2, while leupeptin slowed the generation of intermediate heparan sulfate fragments, it totally prevented the final degradation of these fragments in lysosomes. These data established that the 2 pathways of proteoglycan degradation reside in different intracellular compartments.

Studies have been initiated to investigate proteoglycans in embryonic chick cornea. DEAE-Sephacel and octyl-Sepharose procedures have been developed to purify both the dermatan sulfate proteoglycan and the unique keratan sulfate proteoglycan of this tissue for further structural and biosynthetic analysis.

Bone Cell Biology Section

The activities of the Bone Cell Biology Section center on investigation of the local and systemic control of bone cell differentiation by extracellular bone matrix components. As in previous years the experimental model for these studies is the bone matrix-induced bone development system. In brief, the model consists of implantation of demineralized extracellular bone matrix in a non-skeletal site such that the newly formed bone can be studied by biochemical methods without the complications due to orthotopic skeleton. The current emphasis of the Section is on the purification and characterization of the bone inductive protein(s). Purification of the bovine bone inductive protein was accomplished as follows: One kilogram lots of sized bone matrix was demineralized and then extracted with 4 M guanidine hydrochloride containing a battery of protease inhibitors. Rapid purification of bone inductive proteins was performed by: (1) concentration of the guanidine HCl extracts by ultrafiltration and ethanol precipitation; (2) affinity chromatography on heparin Sepharose and hydroxylapatite; (3) gel chromatography on TSK-3000 by HPLC; and (4) reverse phase HPLC on C-18 columns. This resulted in 3 peaks of activity. One of the peaks consisted of a single silver-stained band with an apparent molecular mass of 20,000 daltons. This material was named osteogenin, the bone inductive protein. Current work is aimed at chemical characterization of the osteogenin molecule at the peptide level. These findings represent a significant achievement in this important area of skeletal biology.

Monoclonal antibodies were raised against the osteogenin-rich TSK-3000 intermediate fraction in order to study the structure and function of osteogenin. The resultant hybridomas were selected, subcloned, propagated and isotyped. Indirect immunofluorescence studies indicated that 3 monoclonal antibodies stained the periosteum of new born rat bone, while 2 others stained cartilage matrix. The blocking ability of the antibodies was examined and three monoclonals inhibited matrix-induced bone formation *in vivo*. One of these blocking antibodies was identical to a periosteum-staining monoclonal described above.

In continuation of ongoing studies on the systemic control of matrix-induced bone formation, the influence of diabetic bone matrix on this process was investigated. In earlier studies it was demonstrated that diabetic rats exhibit decreased levels of matrix-induced bone formation. It was now found that diabetic rat bone matrix is more active in osteoinduction than control matrix. These results may imply a possible compensatory increase in the bone inductive potential of bone matrix in experimental diabetes.

Skeletal Biophysics Section

Crystal Chemistry Program -- National Bureau of Standards

Prior work performed in this program established that artificial lipid vesicles, or liposomes, can be usefully employed as synthetic models for studying matrix vesicle mediated calcification processes in skeletal tissues. During the past year, the versatility of artificial vesicles as model structures for this purpose was enhanced with the observation that liposomes composed of phosphatidylcholine, dicetyl phosphate, and cholesterol (7:2:1) did not in themselves directly initiate calcium phosphate precipitation, but served primarily as membrane-bound vessels for microencapsulating such reactions. Therefore, these liposomes provide a potentially useful means for examining the effect that confinement within a membrane bilayer may have on the ability of various substances to function as biomineralization regulators. For example, phosphatidylserine, a major lipid constituent of matrix vesicle membranes that has received considerable attention as a possible nucleator of apatite *in vivo*, when incorporated into liposomal membranes, was found to actually retard rather than facilitate extraliposomal precipitation in metastable calcium phosphate solutions. Further work will study the influence of other mineralized tissue constituents on the complex chain of reactions associated with matrix vesicle calcification.

The first collaborative research effort to come out of the associate program between the crystal chemistry group of the Skeletal Biophysics Section and the Dental/Medical Materials Group at the National Bureau of Standards was initiated this past year. This joint effort will focus on elucidating factors which delimit the size and influence the shape of apatite crystals grown under physiological-like solution conditions. Initial experiments have been aimed at better understanding the effect of fluoride on apatite crystal growth. Results to date show that fluoride has a much more noticeable impact on crystal growth during the second or consolidating stage of apatite development, where growth is extremely slow, than during the initial formation stage where both the rate of growth and the rate of fluoride incorporation is at a maximum. Fluoride stimulates second stage growth, thereby increasing the rate at which apatite crystals approach their limiting size. These results may partially explain the *in vivo* observation that fluoride-induced improvements in bone apatite crystallinity often lag considerably behind the rate of fluoride uptake by bone mineral.

A comprehensive study to identify changes and probable mechanisms that cause the well-known reduced solubility of laser-irradiated human tooth enamel was completed. An understanding of these mechanisms is important because of the potential clinical use of laser irradiation to reduce caries. Previously identified thermally-induced compositional, structural, and phase changes in enamel, expected to occur in laser-irradiated enamel and experimentally observed in the higher temperature ranges analyzed thus far, were correlated with their probable effects on altering enamel solubility. Other important considerations of laser-irradiant treatment and interactions of enamel considered discussed in this study were: enhanced uptake of fluoride, more effective irradiant wavelengths, selective reactions, directional absorption in crystals, and threshold irradiant conditions.

Connective Tissue Nuclear Magnetic Resonance Program

The effect of mineralization on collagen helix stability was studied by following the rate of ^{1}H - ^{2}H exchange of glycine peptide hydrogens (with D_2O) in samples of collagen specifically enriched with ^{15}N-glycine following injection of this labelled amino acid into growing rats. It was found that the rate of ^{1}H - ^{2}H exchange in rat calvaria was 3-4 times slower than that in rat tail tendon, using solid state nuclear magnetic resonance (nmr) techniques (^{15}N spectra) to examine the isotopically labelled tissues. This result shows that mineralization significantly reduces the rate of ultra-slow helix-opening motions of the collagen molecule and restricts access of water molecules to the interior of the collagen triple helix. The overall effect of mineralization then is to stabilize the collagen helical structure. In other studies of collagen, ^{19}F-labeled (para)L phenylalanine (phe) was used as a "reporter" probe using ^{19}F nmr spectra. Based on these data, it was concluded that (a) 1 out of 13 phe residues per collagen α-chain is very flexible and probably represents motion in the nonhelical carboxyl termini of the collagen chains, and (b) about 6 of the remaining phe residues are reasonably flexible while another 6 are not, only exhibiting molecular motion typical of crystalline parafluorophenylalanine. Thus, intermolecular interactions of phe residues within intact collagen fibers vary significantly along the length of the collagen triple helix. Consequently, complex intermolecular interactions within collagen are to be expected and simple structural models of collagen that assume uniformity throughout the constituent α-chains are not verified by these experimental data.

In other studies, the molecular dynamics of oriented and unoriented DNA fibers were studied using ^{2}H nmr spectra of specifically labelled bases. In contrast with B-DNA, where the base-plane normal was found to be along the fiber axis, that of A-DNA was tilted by almost 20°. These two forms also differed in their molecular mobility. These data improve our knowledge of the differences in structure and dynamics between the A and B forms of DNA fibers. In a separate collaborative effort, ^{13}C spectra of mouse epidermal keratin subunits which were isotopicallaly enriched with ^{13}C-glycine were examined in order to probe various aspects of the hypothesized secondary structure of these intermediate filament molecules. These studies showed for the first time that the glycine-rich N and C terminal domains of the keratin chains are highly flexible and lack significant long range order. This approach should prove useful in determining the key elements operative in intermediate filament structure in tissues.

By direct observation of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ nmr spectra in solutions of cartilage proteoglycans, it was determined that proteoglycans bind these divalent cations to a greater extent than they bind Na^+ or K^+. However, the fraction of calcium bound to the proteoglycans is small relative to that bound to such calcium-binding proteins as calmodulin or staphylococcal nuclease. This observation along with results from ^{13}C nmr of proteoglycans in the presence of calcium, is consistent with Ca^{2+} binding to only a small number of specific sites on proteoglycan molecules. Thus, expectations of extensive calcium binding to proteoglycans based on considerations of composition alone (e.g., sulfation, etc.) are not consistent with these data. To expand our knowledge of calcium-noncollagenous protein interactions in skeletal tissue, a series of model experiments with staph. nuclease (Nase), a calcium dependent phosphodiesterase of known structure, were initiated. A recombinant plasmid under transcriptional control of the bacteriophage λ promotor and constructed to express the structural gene for Nase was used to obtain large quantities of the protein. (This plasmid was derived from the expression vector pCQV2 and carries the gene for a temperature sensitive λ repressor: following thermal denaturation repressor, E. coli can accumulate nearly 50% of its total protein as Nase gene product). Preliminary data demonstrated a tight $^{43}Ca^{2+}$ binding to the recombinant was product that is quite sensitive to the presence of specific inhibitors of the enzyme. Thus, the data show that $^{43}Ca^{2+}$ nmr is a fast, sensitive means for monitoring specific binding of this ion to protein substrates and future data should further test the feasibility of extending this approach to skeletal tissue macromolecules.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00012-23 MTRB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fowler, Bruce O.	Research Chemist	NIDR MTRB

COOPERATING UNITS *(if any)*
Dr. S. Kuroda, Tokyo Medical and Dental Univ., Japan; Dr. J. Christoffersen, Univ. of Copenhagen, Denmark

LAB/BRANCH
Mineralized Tissue Research Branch

SECTION
Skeletal Biophysics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.15	1.00	.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (e.g., hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependency and polarization) are then utilized to establish compositional and structural details of the apatites in question which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-12 MTRB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fisher, Larry W.	Staff Fellow	NIDR MTRB
Termine, John D.	Chief	NIDR MTRB
Tuross, Noreen	NIH Postdoctoral	NIDR MTRB
Bolander, Mark E.	Staff Fellow	NIDR LDBA

COOPERATING UNITS *(if any)*
S.W. Whitson, SIU, School of Dentistry, Edwardsville, IL; S. Weinstein, U. Iowa Med. School; J. Menczel, FIC, NIH; M.A. Drum, CIPC, NIDR; K. Vogel, Univ. New Mexico, Albuquerque, N.M.; M. Bolander, LDBA, NIDR

LAB/BRANCH
Mineralized Tissue Research Branch

SECTION
Skeletal Matrix Biochemistry Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.10	1.60	2.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The extracellular matrix proteins of the bones and teeth are key elements in the structure and metabolism of these tissues. The goal of this project is to study matrix proteins specific to each mineralizing skeletal tissue in order to understand their molecular structure and biological function.

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binding, RIA, ELISA, etc.) have been developed to quantitate the levels of bone specific noncollagenous proteins in (a) surgical specimens of bony tissue (osteonectin, bone sialoprotein and bone proteoglycan) and (b) serum (osteonectin). Changes in the noncollagenous protein profile with age and variety of bone (and tooth) diseases have been observed in man and several animal models. Recent developments in monoclonal antibody production against osteonectin suggest that a family of related proteins similar to bone osteonectin may exist in tissue culture.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00088-12 MTRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical, Structural, and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Eanes, E.D.	Chief, Skeletal Biophysics Section	NIDR MTRB
Heywood, B.	Visiting Fellow	NIDR MTRB
Talwar, H.S.	Visiting Fellow	NIDR MTRB
Hand, A.	Chief, LOBP	NIDR LOBP

COOPERATING UNITS *(if any)*

Dr. Ming Tung, American Dental Association Health Foundation Research Unit, National Bureau of Standards, Gaithersburg, MD

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.85	1.60	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium phosphate salts provide the hardness and rigidity which uniquely characterize normal, healthy bones and teeth. Developmental defects in the deposition of these salts or their destruction and loss by disease can severely impair the function of these skeletal tissues. The purpose of this project is to study the physical, chemical, and ultrastructural properties of these salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable these salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. Topics under current investigation include (1) the formation and properties of precipitates induced in microencapsulated phosphate solutions by the ionophoric translocation of calcium ions across lipophilic solvent barriers, and (2) the growth dynamics of apatite crystals prepared under physiological-like aqueous solution conditions. The ionophore experiments are being conducted with liposomes with the goal of better understanding how matrix vesicles, the loci for initial mineralization in many vertebrate hard tissues, can concentrate to precipitable levels calcium and phosphate in their membrane and interior spaces. The purpose of the crystal growth experiments is to better elucidate the physicochemical and physiological factors delimiting the size and shape apatite crystals can attain in various hard tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00134-11 MTRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Biosynthesis of Proteoglycans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hascall, Vincent C.	Chief, Proteoglycan Chemistry Section	NIDR MTRB
Yanagishita, M.	Visiting Scientist	NIDR MTRB
Morales, T.	Kroc Foundation	NIDR MTRB
Oike, Y.	Visiting Fellow	NIDR MTRB
Midura, R.	Guest Researcher	NIDR MTRB
Xiaoming, Tien	Visiting Fellow	NIDR MTRB
McQuillan, D.	Visiting Fellow	NIDR MTRB

COOPERATING UNITS *(if any)* Dr. J. Kimura, Rush-Presbyterian-St. Lukes Med. Center; Dr. S. Lohmander, U. Lund, Sweden; Dr. B. Caterson, U. West VA; Dr. S. Lamberg, Johns Hopkins Hosp.; Dr. J. Dingle, Strangeways Lab., Cambridge, England; Dr. J. Stevens, U. Alabama (Birmingham); Dr. K. Vogel, U. N. Mexico; Dr. C. Handley, Monash Univ., Australia

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.90	6.55	1.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to study the chemical and physical properties and biosynthesis of proteoglycans in a number of tissue and cell systems. Topics of present interest include: 1) Protein chemistry and immunology of the hyaluronic acid-binding region of proteoglycans from the Swarm rat chondrosarcoma; 2) Biosynthesis of the core protein precursor, and the completed proteoglycan with its associated oligosaccharides and of the link protein by chondrocytes; 3) Metabolism of proteoglycans by rat ovarian granulosa cells; 4) Effects of bacterial endotoxins (lipopolysaccharides) and catabolin on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilage; 5) Characterization of the keratan sulfate-proteoglycan in cornea.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00157-09 MTRB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biophysical Studies on the Structure of Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Torchia, Dennis A.	Biophysicist	NIDR MTRB
Hiyama, Yukio	Visiting Associate	NIDR MTRB
Mack, James W.	Chemist	NIDR MTRB
Sparks, Steven W.	Staff Fellow	NIDR MTRB
Lerner, Laura	Chemist	NIDR MTRB

COOPERATING UNITS *(if any)* Dr. Paul E. Young, York College, SUNY, Jamaica, N.Y.; Dr. Peter Steinert, DB, NCI, NIH; J.T. Gerig, Dept. of Chemistry, Univ. of Calif., Santa Barbara, CA; Dr. J.V. Silverton, LC, NHLBI, NIH; Dr. Attila Szabo, LCP, NIADDK, NIH; Dr. H. Shindo, Tokyo College of Pharmacy, Tokyo, Japan

LAB/BRANCH
Mineralized Tissue Research Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.80	4.00	.80

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to investigate the molecular structure and dynamics of proteins, peptides and proteoglycans. The structural and dynamical information obtained will be correlated with function. Areas of present interest are 1) Molecular structure and dynamics of collagen and DNA fibers. Flourine-19, nitrogen-15 and deuterium nuclear magnetic resonance techniques are being used to study the molecular dynamics and interactions in collagen and DNA fibers and in appropriate model compounds. 2) Proteoglycan dynamics and interactions with cations. Carbon-13, sodium-23, potasium-39, magnesium-25 and calcium-43 nmr spectroscopy are being used to study the interacton of glycosaminoglycan chains with cations in cartilage and in solution. 3) Mouse epidermal keratin subunit. Carbon-13 nmr is being used to study the structure of keratin intermediate filaments obtained from mouse epidermal cells. 4) Calcium binding proteins and peptides. This is a new project in which we are using multinuclear nmr to study the molecular structure and interactions of calcium with (a) staphylococcal nuclease and (b) peptides that transport calcium across cell and model membranes. For these studies, we have assembled state-of-the-art multinuclear magnetic resonance spectrometers which give spectra of solution and solids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00204-08 MTRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Local and Systemic Regulation of Bone Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Reddi, A.H.	Chief, Bone Cell Biology Section,	NIDR MTRB
Kuberasampath, T.	Visiting Associate	NIDR MTRB
Muthukumaran, N.	Visiting Fellow	NIDR MTRB
Kapur, S.	Guest Researcher	NIDR MTRB
Landesman, R.	Guest Researcher	NIDR MTRB

COOPERATING UNITS *(if any)*

Dr. Bellur Prabhakar, LOM, NIDR; Dr. Mark Nathanson, New Jersey Medical School

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Bone Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.30	3.40	1.90

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to investigate local and systemic control of bone development by extracellular matrix components. The experimental model of matrix-induced bone development is the mainstay of this work. Projects currently under study are: 1) Purification and characterization of bone inductive proteins from bovine bone, 2) Monoclonal antibodies to bone inductive proteins, 3) The fracture-healing potential of matrix-induced bone, 4) Influence of diabetic bone matrix on bone induction, and 5) Chemotaxis of muscle derived mesenchymal cells to bone inductive proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00379-02 MTRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bone and Tooth Gene Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Young, Marian F.	Staff Fellow	NIDR MTRB
Termine, John D.	Chief	NIDR MTRB
Shimokawa, H.	Visiting Associate	NIDR MTRB
Day, Agnes A.	Staff Fellow	NIDR MTRB
Findlay, David M.	Visiting Fellow	NIDR MTRB
Robey, Pamela Gehron	Senior Staff Fellow	NIDR MTRB
Bolander, Mark E.	Staff Fellow,	NIDR LDBA
Yamada, Y.	Visiting Scientist	NIDR LDBA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Matrix Biochemistry Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.40	3.10	1.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective in this investigation is to study the biosynthesis of these macromolecules and to understand the regulation of their expression.

The expression of bone and enamel matrix proteins have been studied by constructing recombinant cDNA libraries from ameloblast and bone cell mRNA. cDNA clones encoding several bone and tooth matrix proteins were isolated using expressing DNA vectors and polyclonal antisera directed against individual bone matrix and ameloblast proteins. The clones are being used to study the primary structure and regulation of expression of these genes during development and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00380-02 MTRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolism of bone cells in vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robey, Pamela Gehron	Sr. Staff Fellow	NIDR MTRB
Denholm, Laurence J.	Visiting Fellow	NIDR MTRB
Beresford, Jon N.	Visiting Fellow	NIDR MTRB
Termine, John D.	Chief	NIDR MTRB

COOPERATING UNITS *(if any)*

Drum, M. Ann CIPCB, NIDR; Thomas D. McClain, SB, NCI; de Grange, Donald and Marx, Stephen J. MDB, NIADDK; Whyte, Michael P. MRU, Shriner's Hosp., St. Louis, MO;

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Matrix Biochemistry

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.10	2.55	1.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bone cells (human and bovine) have been utilized to 1) study the responsiveness of the cells to a variety of hormonal and pharmacological factors such as parathyroid hormone and 1,25 dihydroxy vitamin D_3, 2) to study the biosynthesis of extracellular matrix proteins such as collagen, osteonectin and bone proteoglycan, and alterations of matrix production in the disease Osteogenesis Imperfecta, 3) to serve as a source of mRNA and DNA for studies of these proteins at the genomic level.

ANNUAL REPORT OF THE NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Neurobiology and Anesthesiology Branch is concerned with the study of oral-facial sensation, with particular emphasis on mechanisms of pain and the development of new methods for controlling pain in humans. The Branch utilizes anatomical, physiological, behavioral, pharmacological and psychophysical techniques to study neural function as it relates to the processing of sensory signals about the threat of tissue-damaging stimulation. One series of experiments involves correlative morphological, physiological and neurochemical studies of the organization of the medullary and spinal dorsal horns and the identification of putative neurotransmitters involved in sensory transmission. Other experiments employ correlative behavioral and physiological studies to determine the role of different peripheral and central neural populations in pain and temperature discrimination. The clinical component of the Branch develops new methods for measuring and assessing experimental and clinical pain and applies these methods to the study of mechanisms of acute and chronic pain in humans as well as to the evaluation of new techniques for the treatment of acute and chronic pain.

Our studies this year have emphasized the role of neurochemical messengers in the organization of the medullary and spinal dorsal horns and their role in pain transmission. The findings reveal new principles of neural connectivity in pain pathways: individual neurons receive synaptic input from multiple neurochemical mediators and single nerve cells contain more than one neurochemical. We also have furthered our understanding of new pathways in the spinal cord and brain that carry signals about tissue-damaging stimulation. In addition, we have investigated the selective action of opiate drugs in the dorsal horn and the ability of opiates to alter sensory-discriminative capacities in response to noxious or tissue-damaging stimuli. Our animal studies provide a conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions. Human studies have focused on neuroendocrine responses to pain, stress and anxiety, and the development of new methods for the treatment of pain associated with diabetic neuropathy, postherpetic neuralgia, atypical facial pain and myofascial pain of the head and neck. We continue to develop improved methods for assessing acute and chronic pain.

The Branch coordinates the activities of the multi-Institute collaborative program on clinical pain research. This includes studies on cancer pain in children and adults with NCI staff as well as other studies with NIMH, NHLBI, NICHHD and the Clinical Center.

Investigators in the Branch continue to receive recognition for their research accomplishments through invitations to participate in international and national symposia. Dr. Ruda was invited to an international meeting on opiate analgesia in Toulouse and Drs. Bennett, Dionne and Gracely have participated in national symposia on pain and analgesia. Dr. Dubner recently was nominated for President of the American Pain Society and is Scientific Program Chairman for the 1987 meeting of the International Association for the Study of Pain to be held in Hamburg. He also was elected Vice-President of the International Association for the Study of Pain last year.

Research accomplishments of the Branch are presented in more detail below.

New principles of neurochemical function in the medullary and spinal dorsal horns

The classic notion that nerve cells release one transmitter during synaptic excitation or inhibition of other nerve cells has been discarded in recent years based on a new understanding of the role of neuropeptides in brain function. Our recent studies have shed new light on neurotransmitter function in the dorsal and ventral horns of the spinal cord and dorsal horn of the medulla. Although immunocytochemistry has been used to study the nervous system for several years, recent technical innovations have advanced its application especially in multiple label paradigms. These multiple label experiments allow identification of interactions between neuronal elements. One series of multiple label experiments in our laboratory examines the occurrence of different neurotransmitters in individual axonal endings at the light microscopic level. Using antibodies to neurotransmitters made in different species, coexistent neurotransmitters in the same axon can be labeled with different fluorochromes. Other studies employ retrograde transport of a peroxidase label in combination with immunocytochemistry to identify afferents to an identified neuron. The retrograde marker identifies projection neurons while immunocytochemistry identifies the neurotransmitters of multiple afferents to the neuron.

Our previous studies have examined the role of descending axons which use serotonin as their transmitter and originate from neurons in brain stem raphe nuclei. This past year we have extended these studies to investigate the role of brain stem serotonin neurons that contain coexistent substance P in dorsal and ventral horn circuitry. Axons which contained coexistent serotonin and substance P were found throughout the spinal cord although their density varied widely in the different spinal cord laminae. In the superficial laminae, few coexistent axons were detected among the dense terminal plexi of serotonin and substance P immunoreactive fibers. In lamina V, coexistent axons were more numerous than in other dorsal horn laminae. In lamina X, the density of coexistent axons was greater than that of the dorsal horn but fewer than that found in the ventral horn. The motorneuronal cell groups in the ventral horn exhibited the greatest number of serotonin and substance P coexistent axons. This study demonstrates that axons containing both serotonin and substance P represent a very minor component of either serotonin or substance P input to the dorsal horn. Dorsal horn serotonin afferents involved in analgesic mechanisms likely originate from a subpopulation of brain stem raphe neurons which do not contain substance P and thus are distinct from brain stem neurons which contribute the numerous serotonin and substance P coexistent axons to the ventral horn.

We have begun an analysis of brain stem serotonin neurons to further elucidate their role in the modulation of noxious information. Using multiple immunocytochemical markers, serotonin neurons were observed to be contacted by axons which contain dopamine-β-hydroxylase, (the synthesizing enzyme for norepinephrine), substance P, enkephalin or serotonin. These observations demonstrate that serotonin neurons are modulated by a neurochemically diverse population of afferents, supporting the new principle of multiple neurochemical input to neurons. The presence of norepinephrine contacts on serotonin neurons suggests an interaction between these two monoaminergic systems within the raphe nuclei in addition to that which has been demonstrated at spinal cord levels. The differential role of these monoaminergic systems at different central sites warrants further investigation.

These studies are providing details on how chemical messengers localized to the dorsal horn and brain stem are organized. Knowledge of the synaptic circuitry should lead to the development of new pain control agents that alter pain without affecting non-nociceptive systems. The analysis of substance P, enkephalin and serotonin is of particular importance since all of these chemical messengers have been implicated in pain and analgesia.

New long projection systems carrying pain messages to higher brain centers

Last year we first described the physiological properties of a major new projection system from the spinal cord to the midbrain. We recently found that all of these spinomesencephalic neurons respond to noxious stimuli and the vast majority (92%) are activated exclusively by noxious mechanical or noxious mechanical and noxious heat stimuli. The conduction velocities of the axons of these spinomesencephalic neurons were slow and sometimes extended into the unmyelinated range. Using the intracellular horseradish peroxidase method, which provides a "golgi-like" staining pattern of the complete dendritic arbor and often the axon of cells, thirty-five projection neurons were stained well enough to localize their cell bodies with certainty to lamina I. The dendrites of most lamina I projection neurons were confined to lamina I and the overlying white matter. The lamina I projection neurons were of diverse morphological types including small and large cell bodies of various shapes and smooth or spiny dendrites. A few had axon collaterals suggesting that some of these lamina I projection neurons have segmental as well as supraspinal connections.

All of these lamina I projection neurons also appear to receive serotoninergic innervation. Half of the cells received a high density of serotoninergic contacts throughout their dendritic arbors and others received a low density of contacts. As has been demonstrated previously in our laboratory, the serotonergic input was found on dendritic shafts rather than spines. Very spiny neurons received few serotoninergic contacts. Such findings indicate that these neurons are subject to descending brain stem modulation that is likely inhibitory in nature.

A rather unique finding was that lamina I spinomesencephalic neurons project to the midbrain via the dorsolateral column of the spinal cord. Bilateral lesions of the dorsolateral columns at thoracic levels resulted in a 90 percent decrease in the number of retrogradely HRP-labeled lamina I neurons below the lesion. These findings suggest that activation of this system alone may not produce pain since lesions of the anterolateral columns in primates result in analgesia. The termination of this pathway in the parabrachial region of the midbrain also suggests its role in motivational-affective rather than sensory-discriminative aspects of the pain experience.

Another major somatosensory projection in rats, cats, monkeys, and (presumably) humans, arises from neurons concentrated in lamina IV whose axons travel to the medulla in the dorsal columns. These dorsal column postsynaptic neurons (DCPS) have been studied extensively in our laboratory. Our work, and the work of others, indicated that a large subpopulation (∿ 50%) of DCPS neurons in the cat was driven by input from nociceptors and, thus, potentially capable of transmitting information related to the experience of pain. We found, however, that nociceptive DCPS neurons differed from many nociceptive neurons in other somatosensory projection systems in that they were excited only by myelinated nociceptors and not by unmyelinated nociceptors. Work from another laboratory, however, has shown that DCPS neurons in the rat appear to respond to pinch but never respond to noxious heat, suggesting activation by low-threshold mechanoreceptors that are sensitive to skin stretch rather than by nociceptors. In order to clarify this issue, we tested cat DCPS neurons with repeated (sensitizing) noxious heat. We found that nociceptive DCPS neurons responded to noxious heat, but only if the skin was sensitized. Non-nociceptive DCPS neurons that responded to skin stretch never responded to noxious heat, even when the skin was sensitized. These experiments confirm that many DCPS neurons in the cat are driven by nociceptor input and, thus, a true difference exists in the functional organization of the DCPS systems of rats and cats. It will be important to determine whether DCPS neurons in humans are a component of nociceptive pathways. Furthermore, our knowledge of the function of the DCPS projection is likely to have importance in our understanding of the mechanism that underlies the therapeutic efficacy of dorsal column stimulation and related pain therapies.

Behavioral correlates of neural function in the medullary dorsal horn

We have developed a sensory discrimination task to examine whether monkeys can make fine thermal discriminations in the noxious heat range. With the same task, we have been able to study the effects of opiate drugs applied locally in the medullary dorsal horn. In addition, we have studied the role of attentional factors in sensory discrimination.

The monkeys were trained to detect small temperature changes in the noxious range. A trial was initiated when the monkey pressed an illuminated panel button. Subsequently, a contact thermode, positioned on either the upper lip or on the volar surface of the wrist, increased in temperature from a baseline of 38°C to temperatures of 44°, 45°, 46°, or 47°C. After a variable period of 3 to 9 sec the thermode increased an additional 0.1°, 0.2°, 0.4°, or 0.6°C. The monkey received a fruit juice reinforcer for releasing the button within 2 sec of the onset of the second temperature. The detection latency was defined as the amount of time elapsing from the onset of the second temperature until the button was released.

For stimuli applied on the upper lip the detection threshold from a first temperature shift of 44°C varied from 0.4°-0.6°C. As the first temperature shift increased, detection threshold systematically decreased to approximately 0.1°C from a first temperature of 47°C. The median detection latencies were also inversely related to the intensity of the second temperature increase. When the same stimuli were applied to the volar surface of the wrist, the overall detection latencies were slightly longer than those obtained from the face. However, the detection thresholds obtained from the forearm were similar to those obtained from the face.

These findings indicate that monkeys can make very fine discriminations in the noxious heat range, both on their face and hand. It appears that the sensory-discriminative capacities of nociceptive pathways are similar to that of pathways coding touch and innocuous temperature sensations.

The influence of attentional factors on the detection of small incremental noxious thermal stimuli was studied by signaling the detection difficulty of the second temperature increase. Signaling the monkey that a difficult detection was about to occur resulted in a consistent decrease in median detection latency and a decrease in the detection threshold. In addition, the influence of attentional factors was larger for stimuli near threshold and systematically decreased as the second temperature stimulus intensity increased. These data indicate that the detection of small noxious thermal stimuli can be influenced by attentional factors, but that these factors only operate at near threshold stimuli. Such findings can be applied to the development of pain control methods in humans that alter the subject's attention to noxious or painful stimuli.

The ability of monkeys to detect very small increments in noxious heat suggests that there are central nervous system pathways that code such information. We examined the response properties of neurons in the medullary dorsal horn while monkeys performed the task described above. Neuronal activity was correlated with stimulus and behavioral events such as detection latency, temperature increase, button press, etc. We found that both wide-dynamic-range (WDR) neurons that respond to innocuous and noxious stimuli, and nociceptive-specific (NS) neurons that respond only to noxious stimuli encode the intensity of the second small temperature increases. Median detection latencies were inversely related to the magnitude of peak neuronal discharge. However, the monkey's ability to detect near threshold increases in noxious heat was associated with a greater change in peak discharge frequency of WDR as compared to NS neurons. In addition, when equivalent median detection latencies from different first and second temperature levels were compared, corresponding equivalent peak neuronal discharge rates were found for WDR, but not for NS neurons. Finally, the ability to detect near-threshold stimuli was related to the hold time, or the time between panel press and release. Longer hold times resulted in shorter median detection latencies. Peak neuronal discharge also increased as a function of hold time for WDR but not for NS neurons.

These findings support the view that the magnitude of discharge of WDR medullary dorsal horn neurons is a more accurate predictor of behavior related to the perceived intensity of the stimulus than is the magnitude of discharge of NS neurons. WDR neurons appear to accurately code stimulus intensity. NS neurons, that have higher thresholds and do not code intensity near pain threshold, may be more involved in the aversive component of pain.

We identified several thermosensitive medullary dorsal horn projection and non-projection neurons which exhibited characteristic task-related responses. Task-related responses were excitatory and inhibitory responses which were time locked to various events in the task. Most cells which exhibit task-related responses increased their discharge rate in response to the presentation of the trial initiation light, decreased their discharge during panel button press and then once again increased discharge rate during the presentation of the second temperature shift. These inhibitory and excitatory responses occurred only during performance of the task and are related to sensory events that lead to successful completion of the task. These responses are independent of stimulus modality or stimulus parameters and are not correlated with muscle activity recorded from perioral EMG electrodes.

Neurons with task-related activity may be providing a gain control mechanism for somatosensory information that the animal must use for successful completion of the task. Additionally, these responses may be involved in the transmission of behavioral information to motor cortex to facilitate appropriate goal-directed behavior. The task-related responses may serve as an enhancing mechanism for these signals.

It has been proposed that opiates decrease or suppress the unpleasantness of a painful stimulus rather than altering its perceived intensity. However, some recent work suggests that opiates diminish the perceived intensity of acute experimental pain in humans. We examined the effects of morphine sulfate on the monkey's ability to detect temperature increases in the noxious heat range in the behavioral task described above. Morphine was directly microinjected into the medullary dorsal horn in order to check whether morphine exerts its effects early in the central nervous system (e.g., the site of second order pain relay neurons). Unlike previous behavioral studies, we have ruled out that morphine's effects on detection and discrimination of noxious heat are related to motoric factors by requiring the monkeys to respond to a visual stimulus which was difficult to detect. In order to determine whether morphine produces a specific action on pain perception, we studied morphine effects on the detection of an innocuous cooling stimulus.

When microinjected into the medullary dorsal horn, morphine strongly attenuated the monkey's ability to detect temperature increases in the noxious heat range. These effects were dose- and stimulus-dependent and partially antagonized by the systemic administration of the specific opiate receptor antagonist naloxone. The detection of either visual or cooling cues was not modified by any of the doses of morphine. These results show that morphine impairs noxious heat detection without altering motor function, motivational and attentional components or the ability to detect an innocuous thermal stimulus. These data demonstrate that in addition to the suppression of the unpleasantness of a painful stimulus opiates are also able to attenuate the sensory discriminative component of pain in a specific dose- and stimulus-dependent manner. These effects occur early in the central nervous system, at the level of the second order pain relay neurons. The results confirm the critical role that the medullary dorsal horn plays in the monkey's capacity to make fine discriminations in the noxious range.

The Assessment of Experimental and Acute Clinical Pain

The purpose of these human studies are: (1) to develop psychophysical and behavioral models of pain perception that assess the intensity and unpleasantness of experimental and clinical pain sensation and also assess the ability of subjects to judge their perceptual experience; and (2) to use these models to assess the physiological and psychological mechanisms of pain and analgesia, and the efficacy of pharmacological and non-pharmacological methods of pain control.

A new scaling procedure modeled after classical staircase psychophysical procedures has been developed and provides temporal and dose-response information in a single experimental session. One experiment assessed the reliability of this method in assessing pain from contact heat. Results shows that subjects can perform the task reliably and that it distinguishes between stimulus intensities. In a second experiment, fentanyl, a short-acting opiate, was administered in an open design. Fentanyl significantly increased the stimulus intensities required to elicit the same responses. A third experiment compared fentanyl to placebo in a double-blind design. Fentanyl produced detectable analgesia 2 min after administration that reached a peak effect after 11 min.

Additional studies of verbal descriptor measures developed in our laboratory (DDS, Descriptor Differential Scale) have used a new linear-variance model to identify poor performers in patients rating post-operative dental pain for two hours after extraction of a lower third molar. Ninety-three patients completed the DDS one and two hours post surgery. Results show that sensory intensity scales were reliable between times, and that the items were homogenous within each scale. The performance measures identified 15 poor performers on the sensory intensity scale and 22 on the unpleasantness scale. Eliminating these subjects improved reliability and item homogeneity. A second study administered the DDS to postsurgical dental patients 10 min before and after a double-blind intravenous infusion of either fentanyl or a combination of fentanyl and naloxone, an opiate antagonist. Each scale was sensitive to the analgesic effects of fentanyl, and this sensitivity improved after elimination of poor performers.

These studies show that this method can identify subjects who either did not or could not adequately scale their pain magnitude. Eliminating these subjects improved reliability, item homogeneity, and sensitivity to an opiate drug. This measure may provide an important screening tool in expensive and time-consuming clinical trials, and may provide information about the accuracy of pain description in a clinical situation.

Assessment and Treatment of Chronic Pain

We are continuing to evaluate the effects of narcotic analgesics and electrical brain stimulation on clinical and experimental pain in a group of chronic pain patients, some of whom received chronic brain electrode implants for pain relief. These electrodes are placed in brain pathways where they are presumed to activate descending, opiate-related, pain-suppressing systems. Three patients participated in this study this year. Two participated in a preoperative evaluation, and one received an implanted electrode and participated in a postoperative evaluation. Results are similar to those found previously. Morphine significantly reduced the magnitude of clinical pain in the preoperative assessments and this effect was reversed by naloxone. In contrast, the analgesia produced by brain stimulation in the postoperative evaluation was no greater than that observed after sham stimulation. This analgesia was not reversed by the narcotic antagonist naloxone.

The use of deep brain stimulation to control human pain evolved from the findings in animals that electrical stimulation of peri-aqueductal sites activated a descending analgesic system mediated by endogenous opioid-like compounds. The human brain stimulation procedure is assumed also to activate a descending opioid system. The previous findings that the stimulation-produced analgesia does not show an opioid time course and is not reversed by an opiate antagonist suggest that the analgesia is not produced by an opioid mechanism.

Some of our newer clinical trial studies evaluate mechanisms of pain and new treatment plans for deafferentation pain. Diabetic neuropathy pain and shingles pain are chronic deafferentate pain syndromes associated with sensory loss, hyperalgesia and dysesthetic sensations. They are poorly treated with conventional analgesics. We are evaluating the efficacy of tricyclic antidepressant drugs in the treatment of these painful syndromes. These agents are thought to increase the availability of serotonin levels in the brain and may act by influencing descending pain-suppressing pathways. Twenty-nine diabetic neuropathy pain patients completed a crossover study of amitriptyline versus active placebo. Patients' global evaluation after treatment periods indicated that 23 had less pain with amitriptyline, 1 had less pain with placebo and 5 had similar pain with the two treatments. Diary ratings showed amitriptyline superior to placebo in relieving pain in weeks 3 through 6. As intended, the active placebo produced side-effects in most patients - 83%, versus 97% of patients receiving amitriptyline. Psychiatric interview before treatment divided the group into 14 depressed patients and 15 non-depressed patients and Hamilton Depression scale scores supported this division. Amitriptyline was more analgesic than placebo in both non-depressed and depressed subgroups and pain relief was unrelated to improvement in the Hamilton score.

This study provides the best evidence to date that amitriptyline has a specific analgesic effect independent of its antidepressant effects. This will have an impact in clinical practice, because physicians will use the drug with greater confidence and persistence in patients who are not depressed. It will further encourage inquiry by basic and clinical scientists into the mechanism of this analgesia, which may be enhancement of the action of serotonergic and noradrenergic neurons in the brain stem that descend to inhibit discharge of nociceptive projection neurons.

In a similar study in patients with postherpetic neuralgia, amitriptyline was compared to lorazepam and placebo. An interim analysis of the first 12 patients showed that amitriptyline produced pain relief superior to that of lorazepam and inactive placebo from weeks 3 to 6 and progressively increased over that time. These same patients participated in a single-dose study of clonidine, codeine, ibuprofen and placebo. Patients reported a modest reduction in pain with clonidine compared to placebo. This difference was significant on 6 of the 8 pain scales used as well as on a normalized average of all scales. Side-effects were most common with clonidine, followed by codeine, placebo, and ibuprofen. For all drugs combined, relief was greater with treatments during which any side-effect was reported. If only the trials during which side-effects occurred were considered, there were no analgesic differences among the 4 treatments. These results suggest that the high incidence of clonidine side-effects accounted in large part for its demonstrated analgesic effect.

This study demonstrates the role that drug side-effects can play in promoting analgesia. It suggests that when patients detect side-effects, they raise their expectations that a drug will relieve pain and alter their responses to noxious stimuli. This phenomenon, which has not been so clearly documented in the literature, has implications for both analgesic study design (investigators should use placebos with side-effects matched to the study drug) and for clinical practice (mild side-effects may enhance the effectiveness of analgesics).

We have also investigated the effect of amitriptyline in the management of chronic oral-facial syndromes, with an emphasis on those patients with atypical facial pain often involving peripheral nerve damage. Data has been analyzed on 28 patients. High-dose amitriptyline appears to have a significant effect in lowering pain intensity. There is a non-significant trend to improvement in patients on low-dose amitriptyline. This analgesic action appears to have occurred in both depressed and non-depressed patients. These findings are consistent with the results of our studies with diabetic neuropathy and postherpetic neuralgia patients. Amitriptyline appears to be an effective analgesic in deafferentation types of chronic pain where there are lesions or injuries to peripheral nerves directly. More studies are needed to determine the mechanism of action of tricyclic antidepressants in such conditions.

Myofascial pain dysfunction in the oral-facial region (MPD) has been attributed to a variety of causes, but most observers feel the pain and dysfunction result from masticatory muscle spasm. We are evaluating the efficacy of an analgesic, anti-inflammatory agent (ibuprofen) and an anti-spasmodic and anxiolytic agent (diazepam) in the relief of MPD. Patients are included who have pain in the masticatory muscles of at least 3 months duration on a daily or near daily basis. Criteria for MPD include 1) masticatory muscle tenderness; 2) limited opening; 3) click; 4) deviation on opening. Patients with intracapsular disease and severe depression as assessed by psychological evaluation are excluded. Interim analysis of the first 20 patients suggests some efficacy of active drug as compared to placebo. This study will provide insight into the role of muscle tension, anxiety and inflammation in the production of MPD.

Neuroendocrine responses and their relationship to pain, stress and anxiety

The primary objective of this project is to evaluate the modification by drugs of the neurohumoral, psychological and physiological responses to acute pain and apprehension in patients undergoing a stressful surgical procedure, the removal of impacted third molars. Prototype drugs are employed to evaluate the effects of placebo, anti-anxiety agents, opiate agonists and antagonists on these phenomena. These investigations are clarifying the role of drugs in the control of pain and apprehension, as well as providing information on the interaction of drugs on endogenous pain control systems. The secondary objective of this project is to evaluate the release of pituitary beta-endorphin during surgical stress and post-operative pain, the modification of its release by prototype drugs and the relationship between beta-endorphin levels and pain. Parallel investigations are evaluating the effects of exogenous epinephrine administered with local anesthesia on cardiovascular and catecholamine responses to oral surgery.

Oral surgery conducted with local anesthesia alone resulted in elevated levels of beta-endorphin, norepinephrine and anxiety during surgery. Naloxone-treated patients had significantly greater intra-operative beta-endorphin levels and pain as compared to controls. Both fentanyl and diazepam resulted in significantly lower beta-endorphin levels and anxiety as compared to controls. Norepinephrine levels increased significantly in response to surgical stress in all groups except diazepam. The results indicate that opiate antagonists stimulate and agonists suppress the release of beta-endorphin in response to surgical stress, possibly by affecting the patient's perceived level of pain and anxiety. Additionally, the association of intra-operative hyperalgesia with naloxone pre-treatment suggests that endogenous opioid peptides inhibit the perception of intra-operative pain even in the presence of concurrent local anesthesia.

In a separate study, intravenous administration of human corticotropin releasing factor (CRF) produced a significant increase in plasma beta-endorphin levels which remained significantly greater than levels observed in placebo pre-treated patients for 60 minutes following administration. CRF pre-treated patients reported significantly less pain than placebo at 90 and 120 minutes following administration. CRF had no effect on patient reports of anxiety, blood pressure, heart rate, oral temperature or circulating levels of epinephrine and norepinephrine, suggesting that the CRF-analgesia is a specific effect. These findings suggest a role for the pituitary release of beta-endorphin in the response to surgical stress. These investigations are clarifying the role of endogenous mechanisms of pain inhibition. Better understanding of these dynamic processes will likely result in more rational pharmacological approaches to the management of pain.

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October 1, 1984 - September 30, 1985

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-17 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design and Computer Interfacing of Neurophysiologic Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Frederick J.	Electronic Engineer (Instru)	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology & Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects involve the development of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Electronic circuit design, microcomputers and assembly language programming are used in these instruments and the interfacing equipment connected to laboratory or multipurpose computer installations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-11 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985 CT 006102

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacological Modification of Neuroendocrine Responses to Surgical Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Research Dentist	NIDR NA
Hargreaves, Kenneth M.	Postdoctoral Fellow	NIDR NA
Brahim, Jaime	Senior Staff Fellow	NIDR CIPC
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA
Schmidt, Elizabeth A.	Clinical Nurse	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA

COOPERATING UNITS *(if any)*

Goldstein, David G., NHLBI, HE

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.35	.90

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of three related lines of research evaluating 1) the relationship between plasma beta-endorphin levels and pain; 2) the role of exogenous epinephrine administered with local anesthetics on plasma levels of epinephrine and cardiovascular performance; and 3) the clinical efficacy of drugs given to modify the stress of minor surgery. The major effort during the past year has been to examine the relationship between modifying perceived levels of pain and anxiety caused by oral surgery and concommitant levels of beta-endorphin. Results to date indicate that both diazepam and fentanyl suppress stress-induced release of beta-endorphin while naloxone increases release and produces hyperalgesia during oral surgery. Administration of human corticotropin releasing factor resulted in a significant elevation of plasma beta-endorphin which resulted in less postoperative pain in comparison to placebo. These data suggest a relationship between plasma levels of beta-endorphin and pain which may be a logical avenue for further research to design drugs which activate the endogenous pain inhibitory system without the liabilities of present agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-11 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

CT 0060101

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Maixner, William	PRAT Fellow	NIDR NA
Kwilosz, Donna M.	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.95	.70	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are (1) to assess psychophysical methods of experimental pain measurement, i.e., magnitude estimation, category scaling, and cross-modality matching. Pain will be experimentally induced by electrocutaneous, electric tooth pulp, and mechanical heat stimulation; (2) to assess clinical pain measures, such as pain questionnaires and sensory matching methods, in a dental setting; (3) to determine the validity of experimental pain models by comparison of experimental and clinical pain responses; and (4) to evaluate known pharmacological and non-pharmacological pain-control agents.

The recently-developed interactive computer-based scaling method was assessed in a study comparing the influence of the narcotic fentanyl on pain sensation evoked by contact heat. The method was very sensitive to the effects of fentanyl in relation to saline placebo. Fentanyl reduced pain intensity 2 min after administration and reached a peak effect by 11 min.

The studies of a verbal-descriptor clinical pain measure continued with the development of a linear variance measure of scaling performance. Poor performers were identified; eliminating these invalid profiles improved the psychometric properties of the scales and increased their sensitivity to a narcotic analgesic.

A new study quantified the magnitude of descriptor phrases of pleasantness and unpleasantness by ratio-scaling procedures. Preliminary results show that adverbs modify adjectives by relatively constant amounts independent of the adjective, suggesting that verbal scales can be constructed without extensive psychometric development.

A new series of studies is assessing nonpharmacological methods of pain control. Preliminary results show that analgesia to experimental stimuli following hypnotic suggestion varies with the type of experimental measure and assessment method.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00246-08 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Pharmacological Management of Chronic Orofacial Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Singer, Elyse J.	Medical Staff Fellow	NIDR NA
Dionne, Raymond A.	Research Dentist	NIDR NA
Brahim, Jaime	Senior Staff Fellow	NIDR CIPC
Schmidt, Elizabeth A.	Clinical Nurse	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.15	.75	.40

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to evaluate the efficacy of ibuprofen and diazepam alone and in combination, versus a placebo, in the short-term management of patients with the myofascial pain-dysfunction syndrome (MPD). Pain and mandibular function are assessed over a washout period (2 weeks) and treatment period (four weeks). Beta-endorphin levels and cortisol levels are being assessed from blood samples of these patients. An amendment was added in order to assess the usefulness of new diagnostic tests such as CT scanning of the temperomandibular joint in these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00247-08 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytomorphology of Functionally Characterized Spinal Dorsal Horn Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bennett, Gary J.	Senior Staff Fellow	NIDR NA
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NIDR NA
Kamogawa, Hiroyuki	Visiting Fellow	NIDR NA
Sahara, Yoshinori	Visiting Fellow	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.00	1.20	.80

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dorsal column postsynaptic tract neurons were recorded electrophysiologically in the lumbar dorsal horn of anesthetized cats. Pinch-responsive (putatively nociceptive) and pinch-nonresponsive cells were subjected to repeated noxious heat stimuli. Pinch-responsive cells were shown to have nociceptor input from high-threshold mechanoreceptive nociceptors because they responded to noxious heat only when their cutaneous receptive field had been sensitized by previous noxious heat. Pinch-nonresponsive neurons did not respond to noxious heat. These experiments confirm a significant difference between rats and cats in the functional organization of a major somatosensory projection. A double label method was devised to unequivocally locate peptidergic primary afferent axon terminals within the dorsal horn. Preliminary results confirm the presence of primary afferent terminals in laminae I-II which contain substance P-like immunoreactivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00276-07 NA

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brain Stimulation Analgesia in the Control of Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Max, Mitchell B.	Neurologist	NIDR NA
Kwilosz, Donna M.	Psychologist	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA

COOPERATING UNITS *(if any)*
Lees, David E., CC ANES
Young, Ronald, UCLA, Los Angeles, California
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	.70	1.55

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of the study are (1) Assess the effectiveness of chronic electrical stimulation of midbrain sites for the relief of chronic pain in humans; (2) Evaluate the efficacy and mechanisms of traditional narcotic analgesia and compare these to chronic electrical stimulation of midbrain sites; (3) Validate experimental models of pain and their potential diagnostic use in chronic pain patients; and (4) Determine and compare the impact of both traditional narcotic and chronic electrical stimulation therapies on the functional, intellectual and emotional well being of these patients. Participants in this study will be (1) chronic pain patients receiving surgically implanted stimulating electrodes for pain control; (2) chronic pain patients maintained on traditional narcotic analgesics who will not receive implanted stimulating electrodes; and (3) healthy normal volunteers. The effects of chronic brain stimulation in surgical patients will be compared to the effects of narcotics previously administered to patients and to effects of narcotic regimes in nonsurgical chronic pain patients. In addition, the effects of narcotics on perceptual and neural mechanisms of experimentally induced pain will be assessed in pain-free volunteers.

Results from 3 patients this year support previous findings from this project. Morphine significantly decreased the magnitude of low back pain and this effect was reversed by naloxone. The effect of deep brain stimulation was no greater than that observed after sham stimulation and this effect was not reversed by naloxone. The thermal pain studies showed that morphine altered pain sensations mediated by both A-delta and C-fiber primary afferents, and in addition, attenuated the central C-fiber summation observed with delivery of repetitive stimuli delivered at fast rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00286-06 NA

PERIOD COVERED
October 1, 1984 - September 30, 1985

CT 0060133

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Oral Analgesics for Ambulatory Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Research Dentist	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA
Brahim, Jaime	Senior Staff Fellow	NIDR CIPC
Schmidt, Elizabeth A.	Clinical Nurse	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.70	.25	.45

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project consists of studies (1) to evaluate the efficacy of two novel pain control agents, flurbiprofen and etidocaine; (2) to evaluate the interaction of flurbiprofen and etidocaine when given in combination to suppress postoperative pain; (3) to evaluate the ability of a novel non-narcotic analgesic proglumide, to potentiate the analgesic activity of morphine. Both etidocaine and flurbiprofen resulted in greater pain suppression than standard drugs and the combination of the two resulted in greater analgesia than either drug alone. This combination possesses potential for improvement in the treatment of postoperative pain in that 67% of the sample reported little or no pain following the surgical removal of impacted molars, a procedure which normally results in moderate to severe postoperative pain. Proglumide is presently being evaluated in the oral surgery model to test its ability to potentiate morphine analgesia and evaluate its interaction with endogenous pain inhibitory substances such as beta-endorphin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-06 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacological Characterization of Synaptic Circuitry in Dorsal Horn

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ruda, Maryann T.	Research Biologist	NIDR NA
Collantes, Margarita E.	Biologist	NIDR NA
Tashiro, Takashi	Visiting Fellow	NIDR NA
Humphrey, Emma L.	Biol. Lab. Tech. (Elec. Mic.)	NIDR NA
Pretel, R. Stephanie	Postdoctoral Fellow	NIDR NA

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.45	2.50	1.95

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The neural circuitry of the dorsal horn of the spinal cord forms the basis for the mechanisms of pain and analgesia. Our lab has made significant inroads in understanding the neuronal connectivity which subserves these sensory phenomena through experiments involving multiple markers to identify interactions between neural elements.

Coexistence of monoamine and peptide neurotransmitters in axons in the dorsal horn was examined using immunologically distinct multiple fluorescent markers. Coexistence of serotonin and substance P was examined initially because of the important role of serotonin in descending control of nociception. Axons which contained both serotonin and substance P were localized to all laminae of the spinal cord. The number of coexistent axons was greatest in the ventral horn and least in the dorsal horn, especially the superficial layers which are involved in processing nociceptive information. Therefore, brain stem neurons which are the source of spinal cord coexistent serotonin and substance P axons are preferentially involved in modulation of motor rather than sensory systems.

A second series of experiments used the multiple marker immunocytochemical approach in the brain stem raphe neuronal cell groups. Serotonin neurons were found to receive input from norepinephrine, enkephalin, substance P and serotonin axons. These observations suggest that serotonin neurons are modulated by other aminergic systems as well as peptidergic systems. These observations are currently being quantified in an effort to determine the frequency of these inputs.

A third series of experiments relied on the production of monoclonal antibodies to produce distinctive neuronal markers in the dorsal horn. Using brain stem tissue from young rat pups as the antigen, we have produced monoclonal antibodies which identify subsets of neurons in the dorsal horn and brain stem. These markers to the cell surface's of neurons will further our ability to study the organization of dorsal horn and brain stem neural circuitry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH [illegible]VICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-06 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Opiate Administration in the Medullary Dorsal Horn of the Behaving Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Oliveras, Jean-Louis	Visiting Fellow	NIDR NA
Maixner, William	PRAT Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NIDR NA
Bates, Renee	Psychologist	NIDR NA
Thomas, David Alan	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35	1.45	.90

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies morphine's action upon the monkey's abilities to detect noxious heat stimuli applied to the face. Macaque monkeys were trained to detect small temperature increments (0.4°C, 0.6°C or 1.0°C) from a 46°C baseline. The animals also performed an innocuous thermal cooling task in which they detect a 0.5°C decrease from a 38°C baseline. Additionally the monkeys perform a difficult visual detection task. Morphine sulfate was microinjected in the medullary dorsal horn (0.2 microliters in 2 minutes). Morphine reduces in a dose- (1, 3 or 10 micrograms) and stimulus-dependent manner the monkeys' ability to detect 0.4°C, 0.6°C and 1.0°C. These effects were reversed by the specific opiate antagonist naloxone (0.5 mg/kg/i.m.). Morphine did not affect the detection of a small visual stimulus and the 0.5°C decrement in the innocuous cooling range. These data demonstrate that morphine specifically attenuates noxious heat sensory discrimination when directly applied to the medullary dorsal horn, the earliest central relay transmitting noxious information from the face.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-04 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Discrimination of Thermal Stimuli Applied to the Face in Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Maixner, William	PRAT Fellow	NIDR NA
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Thomas, David Alan	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.35	.80	.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project correlates behavioral responses with neural responses of thalamic projection and non-projection neurons in the medullary dorsal horn (trigeminal nucleus caudalis) produced by noxious thermal stimuli in the behaving monkey. Medullary dorsal horn neurons encode thermal discriminative information used by the monkey to perform a thermal detection task. Many medullary dorsal horn neurons encode thermal intensity in a manner which allows the detection of small changes in noxious temperatures. Certain subpopulations of medullary dorsal horn neurons appear to encode thermal intensity information better at near threshold detection levels than other thermally responsive medullary dorsal horn neurons. Some thermally sensitive neurons also respond to other stimuli used by the monkey for the successful completion of the task. This task-related activity occurs in characteristic patterns of excitation and/or inhibition and some neurons which exhibit such activity project to the thalamus. The neurons examined in this study appear to be important in encoding noxious sensory discriminative information. The task-related responses exhibited by some of these neurons may modulate sensory activity and thereby influence the perception of and response to oral-facial pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00366-03 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analgesic Mechanisms in Patients with Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Max, Mitchell B.	Neurologist	NIDR NA
Dionne, Raymond A.	Research Pharmacologist	NIDR NA
Gracely, Richard H.	Research Psychologist	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA

COOPERATING UNITS *(if any)*

Chang, Alfred E., NCI
Schafer, Susan, CC

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.85	1.05	.80

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this work is to evaluate drug therapy in chronic pain syndromes that are considered resistant to known approaches such as pain caused by nerve injury and by advanced cancer.

The first study in this series has shown that amitriptyline relieves diabetic neuropathy pain in patients with normal as well as depressed mood. Twenty-nine patients received 6 weeks of amitriptyline (25-150 mg at bedtime, to maximum tolerated) and 6 weeks of a placebo chosen to mimic amitriptyline side-effects, in a crossover design. Patients' global evaluations after treatment periods indicated that 23 had less pain with amitriptyline, 1 had less pain with placebo, and 5 had similar levels of pain with the two drugs. Amitriptyline was more analgesic than placebo in both the 15 non-depressed patients and in the 14 depressed patients. We conclude that amitriptyline has an analgesic action in diabetic neuropathy that is independent of its antidepressant effects.

In 40 patients with post-herpetic neuralgia, a comparison of single doses of clonidine, codeine, and ibuprofen to placebo did not demonstrate a specific analgesic effect for any drug. It was shown that with drug or placebo, patients report more pain relief when they detect side-effects, which has implications for analgesic trial design and clinical treatment. Thirty-two of a projected 40 post-herpetic neuralgia patients have completed a study of chronic amitriptyline vs. lorazepam vs. placebo; an interim analysis showed that amitriptyline was a superior analgesic to the other two treatments. A study of the delta opiate agonist metkephamid in cancer patients with varying degrees of tolerance at the mu or morphine receptor was suspended because of drug side-effects. Resumption awaits the availability of other investigational delta or kappa receptor opiates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00377-02 NA

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Responses of Primate SI Cortical Neurons to Noxious Temperature Increments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Bates, Renee	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.30	.70	.60

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study examines the ability of monkeys to detect small temperature changes superimposed upon noxious levels of thermal stimulation. Past research suggests that the pain sensing system is a relatively crude, impercise system whose primary function is to signal the presence of potentially tissue damaging stimuli. However, the results of the present study show that monkeys can detect extremely small temperature changes in the pain sensitivity range applied on either the lip or the volar surface of the forearm. These data also suggest that the detection latencies to small incremental temperature shifts in the noxious range provide a measure of the perceived intensity of the sensation. Comparison of the stimulus-response functions obtained from the face with those obtained from the forearm indicate that both areas are equally sensitive to small temperature changes. Furthermore, the sensitivity of the monkey to small temperature changes is not immutable, but rather can be systematically altered by attentional factors.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00378-02 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Amitriptyline in the Relief of Orofacial Pain, Depression and Sleep Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Research Dentist	NIDR NA
Singer, Elyse J.	Medical Staff Fellow	NIDR NA
Schmidt, Elizabeth A.	Clinical Nurse	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA

COOPERATING UNITS *(if any)*

Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.20	.85	.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of amitriptyline in two doses (30 and 150 mg/day) as an analgesic are tested in patients with chronic orofacial pain. The following are evaluated: evaluation of chronic pain intensity and unpleasantness, mood, anxiety level, sleep disturbance, and beta-endorphin level in order to determine 1) if amitriptyline is useful in the management of chronic orofacial pain; 2) the optimum dosage for treatment; 3) to determine if there is an analgesic effect separate from antidepressant activity.

The double blind incomplete cross-over design of drug administration is used and each subject is administered in two four-week periods, separated by a two week washout, one of the following: placebo, high dose amitriptyline, or low dose amitriptyline.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00394-01 NA

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiology and Morphology of Dorsal Horn Projection Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hylden, Janice L.K.	Postdoctoral Fellow	NIDR NA
Bennett, Gary J.	Senior Staff Fellow	NIDR NA
Hayashi, Haruhide	Visiting Associate	NIDR NA
Ruda, Maryann T.	Research Biologist	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Nahin, Richard L.	Postdoctoral Fellow	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	2.35	.65

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurons in lamina I of cat lumbar spinal cord were identified as having projections to the brain by antidromic activation at the rostral cervical spinal cord. Many of these neurons were also found to project to the midbrain (nucleus cuneiformis and lateral periaqueductal gray; n=89), thalamus (both medial and lateral nuclei; n=8), or both the midbrain and thalamus (n=8). The majority of lamina I projection neurons (85% overall and 92% of identified spinomesencephalic and/or spinothalamic neurons) responded exclusively to noxious stimulation of their peripheral receptive fields. Central conduction velocities of lamina I neurons was slow; 0.9 to 26 m/s. Intracellular staining revealed that lamina I projection neurons were of diverse morphological types, but were generally confined to lamina I and the overlying white matter and were oriented in the rostrocaudal direction. Morphological characteristics did not appear to correspond to physiological categories. Immunocytochemical staining of serotonin fibers and varicosities showed that lamina I projection neurons received either a high or low density of serotonergic contacts on dendritic shafts. The degree of innervation did not correspond to response category or identified terminations. Retrograde labeling of spinomesencephalic neurons showed high concentrations of cells in laminae I and V. Bilateral lesions of the dorsolateral funiculi at low thoracic levels resulted in the absence of labeled lamina I cells below the lesion. Local injection of colchicine to block transport of the retrograde marker allowed visualization of fibers bilaterally in the dorsolateral funiculi and in the ipsilateral anterolateral quadrant.

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